CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 2 NOVEMBER, 1942 NUMBER 11

# B Vitamins in Cancerous Tissues

## I. Riboflavin

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(Received for publication June 16, 1942)

The occurrence of the B vitamins in all forms of living matter is strong presumptive evidence of their essential nature for the fundamental vital processes. The functions of many of these vitamins are largely unknown as yet, but the importance of riboflavin in cellular oxidations has been amply demonstrated in recent years (1).

Warburg (17) was the first to show that the oxidation processes in cancerous tissues differed significantly from those in noncancerous tissues, and a number of workers have since confirmed and extended these findings (3, 6, 5). It has therefore become of particular interest to investigate the distribution of the enzymes concerned with cellular oxidations in different types of cancer, and if possible to evaluate the significance of the results in terms of the transformation to the cancerous condition.

Riboflavin has received particular attention in the case of the liver tumors induced by feeding *p*-dimethylaminoazobenzene. It was found that tumors appeared rapidly when a basal diet of rice and carrots (deficient in riboflavin) was used in conjunction with the azo dye, and the tumors contained much less riboflavin than was found in noncancerous rat livers (12, 13, 11). When the diet was supplemented with riboflavin, in combination with other essential substances, the carcinogenic effect of the dye was considerably inhibited although not completely abolished. Greenstein, Jenrette, and White studied the distribution of a particular riboflavin enzyme, xanthine dehydrogenase, in liver tissue and found that transplants of hepatic tumor No. 31 in rats contained only about half of the enzyme present in normal livers (9).

Brabec reported that the use of diets deficient in vitamin G did not prevent takes or growth of sarcoma 39 in rats (2). Analyses of the tumors for this vitamin showed only about one-seventh as much as was found in liver. It was concluded that this low value did not indicate consumption of vitamin G by the tumor since there was only a slight difference in the vitamin contents of the livers of tumor-bearing and non-tumor-bearing rats. Recently Lewisohn and his coworkers (14) obtained more than 60 per cent non-takes of a mouse mammary carcinoma transplant by injections of riboflavin together with yeast extract.

In this paper are reported the results of riboflavin assays on a variety of human and animal tumors, as well as on some typical noncancerous tissues for comparison.

### MATERIALS AND METHODS

The assay method used was that of Snell and Strong (15), wherein the growth responses of *Lactobacillus casei* ϵ to tissue extracts were compared with the responses to graded doses of pure riboflavin.

The tissues were carefully selected to avoid necrotic and extraneous material, and then prepared for test by mincing very finely and hydrolyzing enzymatically by means of 2 per cent added papain and taka-diastase preparations in acetate buffer at pH 4.5. Recent results from this laboratory have shown this method to be effective for the liberation of riboflavin from tissues (4). The tissues were allowed to hydrolyze for 24 hours at 37.5° C. under benzene, and were then steamed and filtered, the filtrates being used for the analyses. All results are reported in micrograms per gram of fresh tissue weight, although they can be converted to a dry weight basis by means of the solids contents listed in the tables.

Tumors of the rat, mouse, and man were included in this study. The rat tumors were of two types, both grown in albino rats of the Wistar strain, and consisted of the Walker carcinosarcoma 256 and chemically induced hepatomas. For the production of liver tumors, one group of rats was maintained on a basal diet of Purina dog chow and a second on a diet of cooked whole rice, raw carrots, and salts. *p*-Dimethylaminoazobenzene to the extent of 0.6 per cent was added to each diet in the form of a 3 per cent solution in olive oil.

The mouse tumors were of three kinds: mammary gland adenocarcinoma transplants maintained in mice of the dba and C3H strains, and methylcholanthrene-induced sarcomas in mice of the C57 strain. The sarcomas were produced by means of a single subcutaneous injection in the right axilla of 0.4 mgm. of methylcholanthrene dissolved in 0.2 ml. of olive oil.

Medium sized tumors were selected for assay, since at this period there was little or no necrotic tissue present and the host animals were still in good condition. Samples of each tumor utilized were prepared for histological study.

Tumor material of human origin was obtained through the courtesy of Dr. C. P. Rhoads of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, N. Y. Each of these specimens was carefully dissected out with the object of obtaining samples for assay which contained a minimum of nonneoplastic tissue. In addition to this, histological sections were prepared and careful study made of the microscopic structure. Tissues from a noncancerous human female were obtained from Dr. A. C. Ivy of Northwestern University and Dr. S. A. Levinson, Cook County Hospital, Chicago.

## HISTOLOGICAL CHARACTERISTICS OF TUMOR SAMPLES

### Rat and Mouse Neoplasms

It is well known, of course, that tumors even of the same type and derived from the same tissue may differ considerably in the amount of nonneoplastic tissue (stroma) present and in the denseness or compactness of the groups of cancer cells. Such characteristics are of special importance in evaluating the results of vitamin assays of tumor tissue. Some estimate of the proportion of a tumor sample occupied by neoplastic cells is especially pertinent in this regard. With these considerations in mind a study was made of each of the tumors reported in Table II.

Samples No. 107, 111, and 279 were liver tissues from rats which had been ingesting *p*-dimethylaminoazobenzene. These samples consisted mainly of nonneoplastic parenchymal cells with scattered lesions, some of which appeared neoplastic in character. Samples No. 107 and 111 were from female rats which had been on the rice-carrot-*p*-dimethylaminoazobenzene diet for 134 days. Sample No. 279 was from a female rat which had been on Purina and *p*-dimethylaminoazobenzene for 290 days.

Samples No. 169, 197, and 280 appeared to fall in the classification hepatoma type II, after the terminology used by Edwards and White (8). Sinuses were a prominent feature. Neoplastic cells accounted for most of the total tissue volume. Sample No. 169 was from a male rat which had been on a rice-carrot-*p*-dimethylaminoazobenzene diet for 199 days; sample No. 197 from a female rat which had been on the Purina-*p*-dimethylaminoazobenzene diet for 210 days; sample No. 280 from a female rat which had been on the Purina-*p*-dimethylaminoazobenzene diet for 290 days.

Samples No. 158, 159, and 160 consisted of tumors from each of 3 rats bearing transplants of the Walker carcinosarcoma 256. This tumor was of a fast growing type and was made up chiefly of neoplastic cells with a relatively scanty stroma.

Samples No. 207, 208, and 209 consisted of transplanted mammary adenocarcinomas from each of 3 female dba mice. These tumors were fast growing and nonhemorrhagic. Histological examination disclosed masses of tumor cells with a scanty stroma.

Samples No. 250, 251, 252, 274, and 275 were of tumor obtained from each of 5 female C3H mice which bore mammary adenocarcinoma transplants. These tumors were slightly slower in rate of growth than the dba mammary carcinoma transplants described above, and a hemorrhagic tendency was present. Microscopic examination disclosed fairly homogeneous masses of tumor cells with a moderate stroma. These samples tended to have a slightly smaller proportion of neoplastic cells than the preceding dba tumors.

Samples No. 176, 180, and 184 were taken from methylcholanthrene-induced tumors grown in C57 female mice. They consisted of fast growing sarcomas, mainly of the spindle cell type. The stroma was negligible, the bulk of the tumor being made up of neoplastic cells.

### Human Neoplasms

Data on the samples of human tissue are contained in Table I.

## RESULTS AND DISCUSSION

On the basis of the riboflavin assays, the normal tissues listed in Table II fall into two groups; namely, those comparatively high and those comparatively low in riboflavin. The former group includes liver, heart, and kidney, while the latter includes brain, lung, spleen, and muscle. There are, of course, differences according to the species of mammal from which the tissues were derived, and diet obviously plays an important part in determining the vitamin levels in tissues. The diet was controlled in all cases except the human subjects, and consisted of Purina dog chow for the animals except where otherwise indicated. The importance of the diet in determining vitamin levels is shown by a comparison of the values for the noncancerous livers of rats on Purina and on the rice-carrot diet (Table IV). The livers of rats on the latter diet (deficient in riboflavin) contained less than half as much riboflavin as the livers of rats on the Purina diet, in agreement with the results reported by Kensler, Sugiura, and Rhoads (12).

The outstanding feature of the results from the cancer samples (Table III) is the comparative constancy of the riboflavin level, regardless of the tissue of derivation. This level is appreciably lower than the levels found for the noncancerous tissues, liver, kidney,

TABLE I: HUMAN TUMOR SAMPLES

| Sample No. | Sex | Age, years | Race | Type of tumor | Density of stroma | Neoplastic to nonneoplastic tissue, rough approximation, per cent |
|---|---|---|---|---|---|---|
| 105 | ♀ | 52 | White | Mammary carcinoma | Medium | 30–40 |
| 106 | ♀ | 51 | " | " " | Heavy | 20–30 |
| 212 | ♀ | 57 | " | Metastatic carcinoma (mammary origin) | Light | 70–80 |
| 213 | ♀ | 71 | " | Mammary carcinoma | Heavy | 20–30 |
| 214 | ♀ | 68 | " | " " | " | 20–30 |
| 215 | ♀ | 58 | Negro | " " | Medium | 30–40 |
| 216 | ♀ | 38 | White | " " | Heavy | 30–40 |
| 217 | ♀ | 58 | " | Metastatic carcinoma (mammary origin) | Light | 70–80 |
| 218 | ♂ | 41 | " | Spindle cell sarcoma | " | 70–80 |
| 219 | ♀ | 77 | " | Epidermoid carcinoma | Heavy | 30–40 |
| 220 | ♀ | 45 | " | Recurrent ovarian adenocarcinoma | Medium | 40–50 |
| 225 | ♀ | 45 | " | Ovarian adenocarcinoma | Heavy | 50–60 |
| 221 | ♂ | 41 | " | Renal adenocarcinoma | Medium | 50–60 |
| 222 | ♀ | 1 | " | Spindle cell sarcoma, axilla | Heavy | 40–50 |
| 223 | ♀ | 77 | " | Melanoma, orbital cavity | Medium | 70–80 |
| 224 | ♂ | 41 | " | Reticulum cell lymphosarcoma, groin | Heavy | 60–70 |
| 281 | ♂ | 73 | " | Melanoma, right lower leg | Medium | 40–50 |
| 286 | ♂ | 58 | " | Recurrent spindle cell myosarcoma | Light | 70–80 |
| 287 | ♀ | 48 | " | Spindle cell metaplastic sarcoma, salivary gland | Medium | 40–50 |
| 288 | ♂ | 66 | " | Neurogenic sarcoma | Heavy | 15–25 |
| 289 | ♀ | 63 | " | Adenoma malignum, sigmoid colon | " | 30–40 |
| 290 | ♂ | 57 | " | Adenocarcinoma, rectum | Light | 40–50 |

TABLE II: RIBOFLAVIN CONTENTS OF NONCANCEROUS TISSUES

| Tissue | Sex | Number of samples | Solids content, per cent | Riboflavin content (γ per gm. fresh tissue) |
|---|---|---|---|---|
| Liver—Wistar rat (rice-carrot diet) | ♀ | 2 | 30.5 | 13 |
| " Wistar rat (Purina diet) | ♀, ♂ | 2 | 30.5 | 28 |
| " C57 mouse | ♀ | 3 | 29.6 | 41 |
| " dba " | ♀ | 3 | 28.4 | 31 |
| " C3H " | ♀ | 3 | 32.3 | 25 |
| " Human | ♀ | 1 | 27.4 | 16 |
| Whole heart—Wistar rat | ♀, ♂ | 2 | 20.6 | 12 |
| " " C57 mouse | ♀ | 3 | 22.2 | 36 |
| " " dba " | ♀ | 3 | 23.7 | 20 |
| " " C3H " | ♀ | 3 | 23.8 | 36 |
| Myocardium—Human | ♀ | 1 | 20.6 | 7.8 |
| Whole brain—Wistar rat | ♀, ♂ | 2 | 21.2 | 3.1 |
| " " C57 mouse | ♀ | 3 | 22.8 | 4.3 |
| " " dba " | ♀ | 3 | 21.9 | 2.4 |
| " " C3H " | ♀ | 3 | 22.2 | 3.4 |
| Cerebrum—Human | ♀ | 1 | 23.8 | 2.1 |
| Lung—Wistar rat | ♀, ♂ | 2 | 19.1 | 4.4 |
| " Human | ♀ | 1 | 21.4 | 1.6 |
| Spleen—Wistar rat | ♀, ♂ | 2 | 20.2 | 3.3 |
| " Human | ♀ | 1 | 22.7 | 3.0 |
| Kidney—Wistar rat | ♀, ♂ | 2 | 22.7 | 28 |
| " Human | ♀ | 1 | 20.3 | 25 |
| Skeletal muscle—Wistar rat | ♀, ♂ | 2 | 24.7 | 1.9 |
| " " Human | ♀ | 1 | 26.0 | 2.0 |

and heart. Most of the cancers contained approximately 2 to 3 γ per gm. of moist tissue, the highest value reported being 7.4 for a rat hepatoma. These values appear particularly characteristic of the animal tumors, which were shown by histologic examination to consist mainly of neoplastic cells; but they must be considered only as giving the proper order of magnitude for many of the human samples, where much of the tissue actually analyzed consisted of nonneoplastic material. However, even in these cases the low values show that the cancers did not contain, and were not growing in a medium of, high riboflavin content.

The low values found indicate further that riboflavin is not directly associated with the high glycolysis shown by cancer cells. The transformation to the cancerous condition with its attendant increase in glycolysis does not appear to require or to be accompanied by an increase in riboflavin content. On the contrary, in the case of hepatoma induced by *p*-dimethylaminoazobenzene there is a real fall in riboflavin content, and it is likely that this is true whenever tissues containing much riboflavin undergo transformation to the cancerous state. One would not expect to observe much change in riboflavin content when brain, lung,

TABLE III: RIBOFLAVIN CONTENTS OF CANCEROUS TISSUES

| Sample No. | Tissue | Sex | Solids content, per cent | Riboflavin content (γ per gm. fresh tissue) |
|---|---|---|---|---|
| 158, 159, 160 | Rat carcinosarcoma (Walker 256) | ♂ | 13.9 | 3.2 (2.3–4.1) |
| 176, 180, 184 | C57 mouse sarcoma (methylcholanthrene) | ♀ | 17.5 | 6.0 (5.5–6.9) |
| 207, 208, 209 | dba mouse adenocarcinoma | ♀ | 18.3 | 3.2 (3.0–3.3) |
| 250, 251, 252, 274, 275 | C3H " " | ♀ | 16.3 | 2.1 (1.7–2.9) |
| 105 | Human mammary carcinoma | ♀ | 35.7 | 4.3 |
| 106 | " " " | ♀ | 27.2 | 1.7 |
| 212 | " " " | ♀ | 19.1 | 2.0 |
| 213 | " " " | ♀ | 16.1 | 5.7 |
| 214 | " " " | ♀ | 34.4 | 1.1 |
| 215 | " " " | ♀ | 16.8 | 2.9 |
| 216 | " " " | ♀ | 35.3 | 1.7 |
| 217 | " " " | ♀ | 18.0 | 2.8 |
| 218 | " spindle cell sarcoma | ♂ | 16.4 | 2.2 |
| 219 | " skin carcinoma | ♀ | 17.8 | 1.6 |
| 220 | " ovarian adenocarcinoma | ♀ | 19.5 | 2.2 |
| 225 | " " " | ♀ | 14.8 | 2.2 |
| 221 | " renal adenocarcinoma | ♂ | 19.6 | 2.0 |
| 222 | " sarcoma, axilla | ♀ | 16.7 | 2.1 |
| 223 | " melanoma, orbit | ♀ | 14.3 | 2.3 |
| 224 | " lymphosarcoma, groin | ♂ | 16.7 | 2.4 |
| 281 | " melanoma, lower leg | ♂ | 18.7 | 1.1 |
| 286 | " sarcoma, back | ♂ | 20.4 | 2.9 |
| 287 | " sarcoma, salivary gland | ♀ | 21.5 | 2.8 |
| 288 | " sarcoma, neck | ♂ | 20.0 | 2.1 |
| 289 | " adenoma malignum, sigmoid colon | ♀ | 17.5 | 2.0 |
| 290 | " adenocarcinoma, rectum | ♂ | 20.0 | 2.5 |

TABLE IV: RIBOFLAVIN CONTENTS OF CANCEROUS AND NONCANCEROUS RAT LIVER TISSUES

| Diet | Days on diet | Sample No. | Tissue | Sex | Solids content, per cent | Riboflavin content (γ per gm. fresh tissue) |
|---|---|---|---|---|---|---|
| *p*-Dimethylaminoazobenzene, rice, carrots | 199 | 169 | Hepatoma | ♀ | 17.6 | 3.4 |
| " " | 134 | 107, 111 | Mixed cancerous and noncancerous tissue | ♀ | 28.4 | 14.7, 18.4 |
| " " | 199 | 170 | Noncancerous liver adjacent to hepatoma | ♀ | (30.5) | 11.9 |
| *p*-Dimethylaminoazobenzene, Purina | 210<br>290 | 197, 280 | Hepatomas | ♀, ♂ | (17.6) | 3.9, 7.4 |
| " " | 290 | 279 | Mixed cancerous and noncancerous tissue | ♀ | (28.4) | 13.3 |
| " " | 210<br>290 | 196, 278 | Noncancerous liver adjacent to hepatoma | ♀, ♂ | (30.5) | 29.3, 24.1 |

spleen, muscle, or other tissue of low riboflavin content became cancerous.

These low riboflavin results are also in line with the oft-stated proposition that cancer tissues have a "deficient" aerobic oxidation system (17, 3, 5, 10). Riboflavin has been shown to be a part of aerobic oxidation systems, and one of the functions of riboflavin enzymes is believed to be the transport of hydrogen from pyridinoprotein enzymes to the cytochrome system, which passes it on to oxygen (1). Any deficiency of one of the necessary enzyme systems would interfere with the normal aerobic oxidation. It has already been shown that one of these necessary enzymes, cytochrome *c*, is low in cancer tissues (7, 10, 16) and it may be that riboflavin is also generally low, although apparently not any lower than in certain normal tissues such as brain, lung, muscle, and spleen.

## SUMMARY

A variety of different human and animal cancers have been analyzed for riboflavin and compared with noncancerous tissues. Most of the cancers were found to contain about 2 to 3 γ of riboflavin per gm. of moist tissue, the lowest value being 1.1 and the highest 7.4. The decrease in riboflavin content in the *p*-dimethylaminoazobenzene-induced transformation of rat liver to hepatoma has been confirmed. The riboflavin contents of cancers appear to be of about the same order of magnitude as those of brain, lung, spleen, and muscle (1.6 to 4.4 γ per gm.) and much below those of liver, heart, and kidney (7.8 to 41 γ per gm.)

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# B Vitamins in Cancerous Tissues

## II. Nicotinic Acid

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(Received for publication June 16, 1942)

The importance of this member of the B vitamin family in human and animal nutrition has been demonstrated many times, often dramatically, in the last few years (4, 3, 14, 12). There is little doubt that noncancerous tissues require a supply of this vitamin for continuation of their essential functions, but practically nothing is known about the requirements of cancerous tissues.

Willheim and Bocobo (17) noted some improvement in the general condition when large doses of nicotinic acid were fed to human patients bearing tumors, and Maisin (10) reported that nicotinic amide feeding was helpful in the inhibition of benzpyrene skin tumors in animals. In the case of liver tumors induced by feeding *p*-dimethylaminoazobenzene to rats on a rice-carrot basal diet, it was shown that nicotinic acid in combination with riboflavin has a protective action (9).

In attempting to judge the importance of nicotinic acid in cancer, it would be helpful to know the content of this vitamin in cancerous tissues and how the values change in the transformation from the noncancerous to the cancerous condition. Here, too, not much is known. von Euler and his coworkers (5) studied the ratio of reduced to oxidized cozymase in different tissues and reported that this ratio was higher for Jensen rat sarcoma than for normal rat muscle. Bernheim and Felsovanyi (1) analyzed rat tissues for coenzymes I and II and found that the Walker carcinosarcoma 256 contained much smaller amounts of the coenzymes than did the noncancerous tissues. They mentioned further that they found only traces in three human carcinomas.

Kensler, Sugiura, and Rhoads (8) determined the effect on the coenzyme I content of rat livers of feeding *p*-dimethylaminoazobenzene, and noted that the concentration was decreased in the damaged livers and fell to still lower levels in the cancers which were produced. Kensler, Dexter, and Rhoads (7) were interested further in determining whether the azo dye or its metabolites had any direct action on enzyme systems involving diphosphopyridine nucleotide, and were able to establish *in vitro* that *p*-phenylenediamine and N,N-dimethyl-*p*-phenylenediamine, both probable metabolites of the azo dye, had a strong inhibiting action on fermentation systems in which coenzyme I was the limiting factor. They called attention to the possibility of correlating this inhibitory action of the metabolites with the carcinogenic potency of the parent azo dye.

In connection with the functions of nicotinic acid in cellular metabolism, it is pertinent that the pyridinoproteins were shown to act directly in many of the fermentative transformations of carbohydrate. Thus they might play an important part in metabolic processes whether or not the metabolites eventually underwent oxidation with oxygen. On the other hand, enzymes such as the flavoproteins and cytochrome *c* appeared to function mainly in the transport of hydrogen from the pyridinoproteins to oxygen, and so might be considered less essential for metabolic systems, such as in cancer, where carbohydrate is transformed largely into lactic acid rather than into carbon dioxide and water. This concept would be in agreement with the low cytochrome *c* (2, 6, 15) and riboflavin (11) contents in cancerous tissues and might lead one to expect comparatively high nicotinic acid values in cancer, although there is unquestionably a great deal yet to learn about the functions of these compounds in living tissues.

### MATERIALS AND METHODS

The tissue extracts described in the first paper of this series (11) were analyzed for nicotinic acid by the microbiological method of Snell and Wright (13). The results for noncancerous tissues are given in Table I and for cancers in Table II.

### RESULTS AND DISCUSSION

The constancy of the nicotinic acid contents of the different tumors is striking. Most of them contained between 13 and 29 γ of nicotinic acid per gm. of fresh tissue, the major exceptions being the two hepatomas produced by feeding rats *p*-dimethylaminoazobenzene on a basal Purina ration, where the values were 38 and 59. Considering the wide variation in nicotinic acid content of noncancerous tissues (Table I), the comparative constancy found for the tumors assumes a real significance.

The absolute level of the vitamin in tumors is low compared with noncancerous tissues. For example, the lowest value for a normal animal tissue was 44 γ per gm. (dba mouse brain), which is appreciably above the range for the cancers. This situation is different from that of riboflavin, which also was found to be low in cancerous tissues but not any lower than in a number of noncancerous tissues (11). The tentative conclusion may therefore be drawn that the transformation to the cancerous condition involves a decrease in the nicotinic acid content. This is most clearly seen in the case of the hepatoma induced by *p*-dimethylaminoazobenzene, where the adjacent noncancerous liver serves as a fairly satisfactory control tissue. It will be seen from Table III that the hepatomas contained about one-third to one-fourth of the nicotinic acid found in the adjacent normal liver.

TABLE I: NICOTINIC ACID CONTENTS OF NONCANCEROUS TISSUES

| Tissue | Sex | Number of samples | Solids content, per cent | Nicotinic acid content (γ per gm. fresh tissue) |
|---|---|---|---|---|
| Liver—Wistar rat (rice-carrot diet) | ♀ | 2 | (30.5) | 174 |
| " Wistar rat (Purina diet) | ♀,♂ | 2 | 30.5 | 178 |
| " C57 mouse | ♀ | 3 | 29.6 | 103 |
| " dba " | ♀ | 3 | 28.4 | 107 |
| " C3H " | ♀ | 3 | 32.3 | 93 |
| " Human | ♀ | 1 | 27.4 | 54 |
| Whole heart—Wistar rat | ♀,♂ | 2 | 20.6 | 123 |
| " " C57 mouse | ♀ | 3 | 22.2 | 126 |
| " " dba " | ♀ | 3 | 23.7 | 114 |
| " " C3H " | ♀ | 3 | 23.8 | 129 |
| Myocardium—Human | ♀ | 1 | 20.6 | 41 |
| Whole brain—Wistar rat | ♀,♂ | 2 | 21.2 | 64 |
| " " C57 mouse | ♀ | 3 | 22.8 | 68 |
| " " dba " | ♀ | 3 | 21.9 | 44 |
| " " C3H " | ♀ | 3 | 22.2 | 56 |
| Cerebrum—Human | ♀ | 1 | 23.8 | 20 |
| Lung—Wistar rat | ♀,♂ | 2 | 19.1 | 51 |
| " Human | ♀ | 1 | 21.4 | 18 |
| Spleen—Wistar rat | ♀,♂ | 2 | 20.2 | 69 |
| " Human | ♀ | 1 | 22.7 | 30 |
| Kidney—Wistar rat | ♀,♂ | 2 | 22.7 | 117 |
| " Human | ♀ | 1 | 20.3 | 33 |
| Skeletal muscle—Wistar rat | ♀,♂ | 2 | 24.7 | 81 |
| " " Human | ♀ | 1 | 26.0 | 50 |

TABLE II: NICOTINIC ACID CONTENTS OF CANCEROUS TISSUES

| Sample No. | Tissue | Sex | Solids content, per cent | Nicotinic acid content (γ per gm. fresh tissue) |
|---|---|---|---|---|
| 158, 159, 160 | Rat carcinosarcoma (Walker 256) | ♂ | 13.9 | 19 (17–20) |
| 176, 180, 184 | C57 mouse sarcoma (methylcholanthrene) | ♀ | 17.5 | 29 (26–35) |
| 207, 208, 209 | dba mouse adenocarcinoma | ♀ | 18.3 | 20 (17–26) |
| 250, 251, 252, 274, 275 | C3H " " | ♀ | 16.3 | 28 (18–42) |
| 105 | Human mammary carcinoma | ♀ | 35.7 | 25 |
| 106 | " " " | ♀ | 27.2 | 27 |
| 212 | " " " | ♀ | 19.1 | 15 |
| 213 | " " " | ♀ | 16.1 | 29 |
| 214 | " " " | ♀ | 34.4 | 21 |
| 215 | " " " | ♀ | 16.8 | 28 |
| 216 | " " " | ♀ | 35.3 | 13 |
| 217 | " " " | ♀ | 18.0 | 22 |
| 218 | " spindle cell sarcoma | ♂ | 16.4 | 29 |
| 219 | " skin carcinoma | ♀ | 17.8 | 18 |
| 220 | " ovarian adenocarcinoma | ♀ | 19.5 | 15 |
| 225 | " " " | ♀ | 14.8 | 27 |
| 221 | " renal adenocarcinoma | ♂ | 19.6 | 18 |
| 222 | " sarcoma, axilla | ♀ | 16.7 | 21 |
| 223 | " melanoma, orbit | ♀ | 14.3 | 23 |
| 224 | " lymphosarcoma, groin | ♂ | 16.7 | 26 |
| 281 | " melanoma, lower leg | ♂ | 18.7 | 11 |
| 286 | " sarcoma, back | ♂ | 20.4 | 28 |
| 288 | " sarcoma, neck | ♂ | 20.0 | 12 |
| 289 | " adenoma malignum, sigmoid colon | ♀ | 17.5 | 24 |
| 290 | " adenocarcinoma, rectum | ♂ | 20.0 | 30 |

This result is in agreement with the decrease in coenzyme I reported for the induction of this type of cancer (8).

tion is not clear. The comparative constancy of the vitamin level in tumors may be an indication of the essential nature of the vitamin for cancer metabolism.

TABLE III: NICOTINIC ACID CONTENTS OF CANCEROUS AND NONCANCEROUS RAT LIVER TISSUES

| Diet | Days on diet | Sample No. | Tissue | Sex | Solids content, per cent | Nicotinic acid content (γ per gm. fresh tissue) |
|---|---|---|---|---|---|---|
| *p*-Dimethylaminoazobenzene, rice, carrots | 199 | 169 | Hepatoma | ♀ | 17.6 | 24 |
| " " | 134 | 107, 111 | Mixed cancerous and noncancerous tissue | ♀ | 28.4 | 144, 121 |
| " " | 199 | 170 | Noncancerous liver adjacent to hepatoma | ♀ | (30.5) | 100 |
| *p*-Dimethylaminoazobenzene, Purina | 210<br>290 | 197, 280 | Hepatomas | ♀, ♂ | (17.6) | 38, 59 |
| " " | 290 | 279 | Mixed cancerous and noncancerous tissue | ♀ | (28.4) | 83 |
| " " | 210<br>290 | 196, 278 | Noncancerous liver adjacent to hepatoma | ♀, ♂ | (30.5) | 127, 147 |

In view of the considerations presented in the introduction whereby one might be led to expect high nicotinic acid contents in tumors, it is interesting to speculate on the significance of the finding that this vitamin is actually decreased in cancer. Since the transformation to the cancerous state is accompanied by an increased ability of the cells to metabolize carbohydrate glycolytically (16), the most obvious conclusion is that nicotinic acid is not associated with the increased glycolysis. On the other hand, the constancy of the nicotinic acid content in various tumors is an indication that this vitamin is essential for at least some aspects of cancer cell metabolism. It may be that the cancer cell is able to utilize its nicotinic acid much more efficiently in glycolytic processes than are noncancerous tissues. If this should be the case, it would account for the apparent anomaly of low nicotinic acid content and high glycolysis. The low vitamin level might simply be a reflection of low storage capacity of the cancer cell. These questions await further investigation.

## SUMMARY

The results of nicotinic acid assays on a variety of cancerous and noncancerous tissues are reported. Whereas values ranging from 18 to 178 γ per gm. of fresh tissue were found for noncancerous tissues, the range for the cancers was from 13 to 59 γ per gm., with most of the values falling between 18 and 29 γ per gm.

It is concluded that the transformation to the cancerous state involves a decrease in nicotinic acid content, although whether this signifies low utilization of this vitamin or low storage capacity and efficient utiliza-

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# B Vitamins in Cancerous Tissues

## III. Biotin

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(Received for publication June 16, 1942)

Considerable interest has recently been attached to biotin as a possible essential catalyst for cancerous processes. For many years investigators have been searching for a substance or substances which would be peculiar to cancer or, failing this, which would be more prominent in cancerous tissues than in noncancerous ones. Kögl reported (7) that the proteins of cancerous tissues were high in the "unnatural" (*d-*) form of glutamic acid, but this has since been disputed by many workers (9, 6, 17, 4). Recent investigations of the respiratory enzyme and vitamin contents of tumors showed the existence of quantitative differences from noncancerous tissues (10, 13, 3, 1, 12), but in these cases the general findings appeared to be that tumors were poorer rather than richer in the test substances. West and Woglom (16) recently reported, however, that a number of tumors were richer in biotin than were corresponding normal tissues. This article was followed by a speculation by Laurence (8) that malignant cells require excess biotin for their metabolic activities and that the creation of biotin deficiencies might lead to regression of cancers. Recently, du Vigneaud and his associates (2) studied the biotin content of the diet in relation to the production of rat liver tumors by feeding *p*-dimethylaminoazobenzene, and observed that the addition of biotin to protective diets led to the breakdown of the protection and to the earlier appearance of tumors. The adjective "procarcinogenic" was applied to this effect, although the investigators stated that the mechanism was still obscure.

West and Woglom more recently reported experiments on the effect of biotin deficiency on tumor growth (15). They maintained mice on diets containing avidin, a protein which has been shown to take up biotin rapidly and render it unavailable as a food factor (5), until the animals were within 2 to 3 weeks of death, and then inoculated them with fragments of sarcomas 37 or 180. It was anticipated that the tumors would not grow in this state of biotin deficiency, but instead rapid growth was actually observed. Thus the metabolic processes of these tumors appeared to be independent of a source of biotin. Moreover, these tumors seemed unable to synthesize biotin, for West and Woglom found that they contained much less biotin than corresponding control tissues; and we may conclude temporarily, in these cases at least, that excess biotin is not necessary for the cancerous process. Probably a more significant test would be the effect of biotin deficiency on the growth of tumors which have been shown by West and Woglom to contain much more biotin than corresponding control tissues.

West and Woglom made a strong point of selecting good control tissues as well as good cancer tissues; so that the differences in biotin values between them would be representative of the cancerous transformation in the particular tissues. Under these conditions their finding that some tumors were richer, others poorer in biotin than the corresponding control tissues, may indicate one of at least three things: (a) that changes in biotin content are without special significance in regard to the cancerous transformation; (b) that there may be at least two different types of cancer metabolism, namely that which requires more and that which requires less biotin than the tissues of derivation; and (c) that there may be one main type of metabolism common to all cancer, but that the biotin requirement may be more directly affected by some secondary aspect of the tissue such as that relating to its particular function in the noncancerous, integrated organism. West and Woglom noted that the biotin contents of tumor tissues deviated from the corresponding normal adult levels generally in the same direction as the corresponding embryo tissues, and suggested that this might be significant, possibly being connected with the fact that both tumors and embryo tissues show higher glycolysis than noncancerous adult tissues. Since, however, high glycolysis is associated with both high and low biotin contents, it is very unlikely that there are a direct relationship *and* a common metabolism.

In this paper are reported the results of a number of biotin assays on various human and animal tumors. Since the selection of really good control tissues for comparison is so difficult, we have made no particular effort to do this except in the case of liver cancer (Table IV), presenting instead a general comparison with many noncancerous body tissues.

### MATERIALS AND METHODS

The biotin contents of the tissue extracts were determined by the microbiological method of Snell, Eakin, and Williams (11), which employs the Fleischmann's baker strain of *Saccharomyces cerevisiae*.

The tissue extracts used were the same as those given in the first paper (10). The method of liberation of biotin involved enzymatic hydrolysis by means of papain and taka-diastase. Previous studies in this laboratory (14) have shown that enzymatic hydrolysis is almost, if not quite, as effective as acid hydrolysis. To test the relative efficiency of the two methods in the instances of the cancer samples, several were hydrolyzed in both ways and the biotin contents of the resulting extracts compared (Table I).

It can be seen that the agreement between the two methods is satisfactory, and that enzymatic hydrolysis can be relied upon to give representative values of the total biotin content. Further evidence of this is provided by the agreement between the values reported

by us and by West and Woglom (16, 15) for noncancerous tissues. In comparing these and other values, it should be borne in mind that their results

TABLE I: COMPARISON OF ENZYMATIC AND ACID HYDROLYSIS OF CANCER SAMPLES

| Sample No. | Tissue | Biotin content (mγ per gm. fresh tissue) Enzymatic hydrolysis | Acid hydrolysis |
|---|---|---|---|
| 212 | Human breast carcinoma | 50 | 48 |
| 213 | " " " | 87 | 90 |
| 215 | " " " | 39 | 68 |
| 216 | " " " | 47 | 46 |
| 218 | " spindle cell sarcoma | 86 | 68 |
| 219 | " skin carcinoma | 20 | 55 |
| 220 | " ovarian carcinoma | 13 | 20 |
| 221 | " renal carcinoma | 8 | 15 |

are reported in millimicrograms per gram of dry tissue, and ours in terms of wet tissue. Interconversion of units can easily be carried out by the use of the solids contents given in our tables.

## RESULTS AND DISCUSSION

The biotin contents of noncancerous tissues are summarized in Table II. It will be seen that in general values for the same organ in different animals were of approximately the same order of magnitude: liver being richest, kidney next, then heart, followed by brain, lung, spleen, and muscle. If we compare our results for cancerous tissues with these, we observe that none of the values is as high as those for kidney or liver, and most are lower than those for heart. The biotin level of cancerous tissues appears to be approximately of the same order as those of brain, lung, spleen, and muscle. In the case of hepatoma induced by *p*-dimethylaminoazobenzene, which can be compared with a fairly good control tissue (adjacent noncancerous liver), it is clear that the cancer contained much less biotin than the control tissue, which confirms previous findings (16, 11).

In none of our results were there any indications that cancerous tissues may be rich in biotin. On the contrary, as mentioned above, all our values for cancerous tissues were of the same order of magnitude as the relatively poor (compared with liver, kidney, and heart) noncancerous tissues. From our results we would conclude that the cancerous process is not associated with high biotin, which agrees with the finding of West and Woglom that the growth of mouse sarcomas was not inhibited by a deficiency of biotin. Further indication of this is provided by the fact that, although the Shope rabbit papilloma is much richer in biotin than normal rabbit skin, the transformation of the papilloma into a carcinoma is not attended by a further increase in biotin content (15).

TABLE II: BIOTIN CONTENTS OF NONCANCEROUS TISSUES

| Tissue | Sex | Number of samples | Solids content, per cent | Biotin content (mγ per gm. fresh tissue) |
|---|---|---|---|---|
| Liver—Wistar rat (rice diet) | ♀ | 2 | (30.5) | 1,130 |
| " Wistar rat (Purina diet) | ♀, ♂ | 2 | 30.5 | 840 |
| " C57 mouse | ♀ | 3 | 29.6 | 1,160 |
| " dba " | ♀ | 3 | 28.4 | 860 |
| " C3H " | ♀ | 3 | 32.3 | 840 |
| " Human | ♀ | 1 | 27.4 | 620 |
| Whole heart—Wistar rat | ♀, ♂ | 2 | 20.6 | 390 |
| " " C57 mouse | ♀ | 3 | 22.2 | 160 |
| " " dba " | ♀ | 3 | 23.7 | 230 |
| " " C3H " | ♀ | 3 | 23.8 | 250 |
| Myocardium—Human | ♀ | 1 | 20.6 | 170 |
| Whole brain—Wistar rat | ♀, ♂ | 2 | 21.2 | 100 |
| " " C57 mouse | ♀ | 3 | 22.8 | 81 |
| " " dba " | ♀ | 3 | 21.9 | 87 |
| " " C3H " | ♀ | 3 | 22.2 | 76 |
| Cerebrum—Human | ♀ | 1 | 23.8 | 31 |
| Lung—Wistar rat | ♀, ♂ | 2 | 19.1 | 78 |
| " Human | ♀ | 1 | 21.4 | 19 |
| Spleen—Wistar rat | ♀, ♂ | 2 | 20.2 | 73 |
| " Human | ♀ | 1 | 22.7 | 40 |
| Kidney—Wistar rat | ♀, ♂ | 2 | 22.7 | 790 |
| " Human | ♀ | 1 | 20.3 | 580 |
| Skeletal muscle—Wistar rat | ♀, ♂ | 2 | 24.7 | 52 |
| " " Human | ♀ | 1 | 26.0 | 21 |

The cancers which have been reported to contain more biotin than corresponding control tissues (16, 15) would appear to be more susceptible to curative efforts, inasmuch as the tumors might be more affected by a biotin deficiency than the other body tissues. This phase of the work should be thoroughly investigated.

Table III: Biotin Contents of Cancerous Tissues

| Sample No. | Tissue | Sex | Solids content, per cent | Biotin content (mγ per gm. fresh tissue) |
|---|---|---|---|---|
| 158, 159, 160 | Rat carcinosarcoma (Walker 256) | ♂ | 13.9 | 30 (20–38) |
| 176, 180, 184 | C57 mouse sarcoma (methylcholanthrene) | ♀ | 17.5 | 70 (51–94) |
| 207, 208, 209 | dba mouse adenocarcinoma | ♀ | 18.3 | 130 (100–160) |
| 250, 251, 252, 274, 275 | C3H " " | ♀ | 16.3 | 166 (140–200) |
| 105 | Human mammary carcinoma | ♀ | 35.7 | 43 |
| 106 | " " " | ♀ | 27.2 | 36 |
| 212 | " " " | ♀ | 19.1 | 50 |
| 213 | " " " | ♀ | 16.1 | 87 |
| 214 | " " " | ♀ | 34.4 | 97 |
| 215 | " " " | ♀ | 16.8 | 39 |
| 216 | " " " | ♀ | 35.3 | 47 |
| 217 | " " " | ♀ | 18.0 | 62 |
| 218 | " spindle cell sarcoma | ♂ | 16.4 | 86 |
| 219 | " skin carcinoma | ♀ | 17.8 | 20 |
| 220 | " ovarian adenocarcinoma | ♀ | 19.5 | 13 |
| 225 | " " " | ♀ | 14.8 | 5 |
| 221 | " renal adenocarcinoma | ♂ | 19.6 | 8 |
| 222 | " sarcoma, axilla | ♀ | 16.7 | 55 |
| 223 | " melanoma, orbit | ♀ | 14.3 | 27 |
| 224 | " lymphosarcoma, groin | ♂ | 16.7 | 24 |
| 281 | " melanoma, lower leg | ♂ | 18.7 | 30 |
| 286 | " sarcoma, back | ♂ | 20.4 | 42 |
| 287 | " sarcoma, salivary gland | ♀ | 21.5 | 57 |
| 288 | " sarcoma, neck | ♂ | 20.0 | 19 |
| 289 | " adenoma malignum, sigmoid colon | ♀ | 17.5 | 51 |
| 290 | " adenocarcinoma, rectum | ♂ | 20.0 | 71 |

Table IV: Biotin Contents of Cancerous and Noncancerous Rat Liver Tissues

| Diet | Days on diet | Sample No. | Tissue | Sex | Solids content, per cent | Biotin content (mγ per gm. fresh tissue) |
|---|---|---|---|---|---|---|
| *p*-Dimethylaminoazobenzene, rice, carrots | 199 | 169 | Hepatoma | ♀ | 17.6 | 75 |
| " " | 134 | 107, 111 | Mixed cancerous and noncancerous tissue | ♀ | 28.4 | 390, 400 |
| " " | 199 | 170 | Noncancerous liver adjacent to hepatoma | ♀ | (30.5) | 510 |
| *p*-Dimethylaminoazobenzene, Purina | 210<br>290 | 197, 280 | Hepatomas | ♀, ♂ | (17.6) | 83, 200 |
| " " | 290 | 279 | Mixed cancerous and noncancerous tissue | ♀ | (28.4) | 320 |
| " " | 210<br>290 | 196, 278 | Noncancerous liver adjacent to hepatoma | ♀, ♂ | (30.5) | 770, 890 |

## SUMMARY

The results of a series of biotin assays on a number of different cancerous and noncancerous tissues are reported. The lowest value found was 5 mγ per gm. of fresh tissue. These values are of the same order of magnitude as those for noncancerous brain, lung, muscle, and spleen, much below those for liver and kidney, and slightly below those for heart. None of the cancers studied can therefore be classified as a biotin-rich tissue, and it is indicated that probably cancers neither particularly require nor are associated with a high biotin content.

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# B Vitamins in Cancerous Tissues

## IV. Pantothenic Acid

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Pantothenic acid is one of the newer members of the B vitamin family, and little is yet known concerning its role in growth and metabolism. It has been shown to be an essential growth factor for many different organisms (1, 3, 4, 6, 7, 12, 13, 14, 16, 17, 19, 20) and the minuteness of the requirements makes it highly probable that pantothenic acid functions in enzyme systems.

That cancer cells are characterized by a high fermentation and low R.Q. has been known for many years. Recent investigations of the enzyme systems in cancer tissues have not demonstrated that any of the enzymes studied were significantly increased in accord with the greater fermentative activity of the cancer cells. Nicotinic acid, for example, which is known to be an important part of many enzymes concerned with metabolic transformations of carbohydrate, has been shown to be low, rather than high, in cancer tissues (15). Similarly, the results indicate that the increased glycolysis of cancer tissues cannot be associated with high riboflavin (10) or high biotin (9) content. This laboratory is embarked upon a program which has for one of its main objectives the study of the roles which may be played by the B vitamins in cancer, and the distribution of pantothenic acid is the particular concern of this paper. The fact that there are indications that pantothenic acid may function as a catalyst in carbohydrate metabolism (11, 18) makes it particularly urgent that we survey its role in cancer, where the carbohydrate metabolism differs from that of noncancerous tissues.

Since pantothenic acid is so new, it is not surprising that there is little in the cancer literature about it. Morris and Lippincott (5) studied the effect of pantothenic acid deficiency on the growth of spontaneous mammary carcinoma in C3H mice and found that the tumor growth rate was retarded. This indicated that the tumors actually require the vitamin for growth and was confirmed by the resumption of tumor growth when pantothenic acid was added to the diet. If the pantothenic acid requirement of the tumor should be sufficiently great, then a proper deficiency of this element in the diet might retard or completely inhibit the growth of the tumor without seriously interfering with the metabolic activities of the host. However, Morris and Lippincott concluded that the pantothenic acid deficiency interfered so seriously with the host's nutrition that the procedure was without practical application in tumor therapy.

In an interesting series of experiments, Lewisohn and his coworkers (2) recently reported that the injection of calcium pantothenate along with yeast in mice of the RIII strain led to a considerable reduction in the number of takes of mammary carcinoma 2163 transplants. The percentage of non-takes reported was 48 as compared with 20 for the yeast alone and 5 for the control mice.

One of the methods of gaining some indication of the importance of pantothenic acid in cancerous tissues is to analyze the tumors for the vitamin and compare the results with those from noncancerous tissues. We are not aware that this has been done before, and we are herewith presenting our results to date.

### MATERIALS AND METHODS

The assay method employed was that of Pennington, Snell, and Williams (8), which depends on the growth responses of *Lactobacillus casei* ε. The tissues used and the method of preparing the extracts were described in a previous paper (10).

### RESULTS AND DISCUSSION

From Table I the noncancerous tissues can be classified in the following order of decreasing pantothenic acid content: liver, heart, kidney, and brain, followed by lung, spleen, and muscle at about the same level. A comparison with the results from the cancerous tissues (Table III) shows that almost all the human and rat cancers fall, in pantothenic acid content, into the lung-spleen-muscle class, while the mouse tumors are appreciably higher, corresponding more with brain and kidney.

In so far as the human and rat cancers are concerned, it appears that we have here another example of a vitamin which is not particularly prominent in cancerous tissues. The story is quite clear in the case of the rat hepatoma induced by *p*-dimethylaminoazobenzene (Table III), where three cancers contained 11, 12, and 16 γ of pantothenic acid per gm. of tissue as compared with 95, 79, and 46 γ per gm. for the corresponding adjacent noncancerous livers. Obviously the transformation to the cancerous condition was here accompanied by a considerable decrease in pantothenic acid content. At least part of this undoubtedly results from loss of storage capacity, but it does appear that the hepatoma can get along with comparatively small amounts of pantothenic acid.

### SUMMARY

Various human and animal cancers have been analyzed for pantothenic acid, and the results are compared with those from noncancerous tissues. It is shown that a large drop in vitamin content occurs in the transformation of rat liver to hepatoma by feed-

TABLE I: PANTOTHENIC ACID CONTENTS OF NONCANCEROUS TISSUES

| Tissue | Sex | Number of samples | Solids content, per cent | Pantothenic acid content (γ per gm. fresh tissue) |
|---|---|---|---|---|
| Liver—Wistar rat (rice diet) | ♀ | 2 | (30.5) | 79 |
| " Wistar rat (Purina diet) | ♀,♂ | 2 | 30.5 | 112 |
| " C57 mouse | ♀ | 3 | 29.6 | 83 |
| " dba " | ♀ | 3 | 28.4 | 71 |
| " C3H " | ♀ | 3 | 32.3 | 69 |
| " Human | ♀ | 1 | 27.4 | 31 |
| Whole heart—Wistar rat | ♀,♂ | 2 | 20.6 | 35 |
| " " C57 mouse | ♀ | 3 | 22.2 | 43 |
| " " dba " | ♀ | 3 | 23.7 | 46 |
| " " C3H " | ♀ | 3 | 23.8 | 50 |
| Myocardium—Human | ♀ | 1 | 20.6 | 16 |
| Whole brain—Wistar rat | ♀,♂ | 2 | 21.2 | 12 |
| " " C57 mouse | ♀ | 3 | 22.8 | 28 |
| " " dba " | ♀ | 3 | 21.9 | 21 |
| " " C3H " | ♀ | 3 | 22.2 | 23 |
| Cerebrum—Human | ♀ | 1 | 23.8 | 13 |
| Lung—Wistar rat | ♀,♂ | 2 | 19.1 | 9.0 |
| " Human | ♀ | 1 | 21.4 | 5.0 |
| Spleen—Wistar rat | ♀,♂ | 2 | 20.2 | 9.0 |
| " Human | ♀ | 1 | 22.7 | 5.4 |
| Kidney—Wistar rat | ♀,♂ | 2 | 22.7 | 34 |
| " Human | ♀ | 1 | 20.3 | 16 |
| Skeletal muscle—Wistar rat | ♀,♂ | 2 | 24.7 | 5.0 |
| " " Human | ♀ | 1 | 26.0 | 10 |

TABLE II: PANTOTHENIC ACID CONTENTS OF CANCEROUS TISSUES

| Sample No. | Tissue | Sex | Solids content, per cent | Pantothenic acid (γ per gm. fresh tissue) |
|---|---|---|---|---|
| 158, 159, 160 | Rat carcinosarcoma (Walker 256) | ♂ | 13.9 | 5.2 (4.5–5.5) |
| 176, 180, 184 | C57 mouse sarcoma (methylcholanthrene) | ♀ | 17.5 | 23 (12–30) |
| 207, 208, 209 | dba mouse adenocarcinoma | ♀ | 18.3 | 24 (20–30) |
| 250, 251, 252, 274, 275 | C3H " " | ♀ | 16.3 | 39 (31–47) |
| 105 | Human mammary carcinoma | ♀ | 35.7 | 6.5 |
| 106 | " " " | ♀ | 27.2 | 3.0 |
| 212 | " " " | ♀ | 19.1 | 5.5 |
| 213 | " " " | ♀ | 16.1 | 7.2 |
| 214 | " " " | ♀ | 34.4 | 7.9 |
| 215 | " " " | ♀ | 16.8 | 11 |
| 216 | " " " | ♀ | 35.3 | 6.9 |
| 217 | " " " | ♀ | 18.0 | 7.9 |
| 218 | " spindle cell sarcoma | ♂ | 16.4 | 7.7 |
| 219 | " skin carcinoma | ♀ | 17.8 | 4.0 |
| 220 | " ovarian adenocarcinoma | ♀ | 19.5 | 4.5 |
| 225 | " " " | ♀ | 14.8 | 5.0 |
| 221 | " renal adenocarcinoma | ♂ | 19.6 | 4.4 |
| 222 | " sarcoma, axilla | ♀ | 16.7 | 9.5 |
| 223 | " melanoma, orbit | ♀ | 14.3 | 3.7 |
| 224 | " lymphosarcoma, groin | ♂ | 16.7 | 5.4 |
| 281 | " melanoma, lower leg | ♂ | 18.7 | 4.5 |
| 286 | " sarcoma, back | ♂ | 20.4 | 4.7 |
| 288 | " sarcoma, neck | ♂ | 20.0 | 2.8 |
| 289 | " adenoma malignum, sigmoid colon | ♀ | 17.5 | 7.3 |

TABLE III: PANTOTHENIC ACID CONTENTS OF CANCEROUS AND NONCANCEROUS RAT LIVER TISSUES

| Diet | Days on diet | Sample No. | Tissue | Sex | Solids content, per cent | Pantothenic acid content (γ per gm. fresh tissue) |
|---|---|---|---|---|---|---|
| *p*-Dimethylaminoazobenzene, rice, carrots | 199 | 169 | Hepatoma | ♀ | 17.6 | 11 |
| " " | 134 | 107, 111 | Mixed cancerous and noncancerous tissue | ♀ | 28.4 | 46, 37 |
| " " | 199 | 170 | Noncancerous liver adjacent to hepatoma | ♀ | (30.5) | 95 |
| *p*-Dimethylaminoazobenzene, Purina | 210<br>290 | 197, 280 | Hepatomas | ♀, ♂ | (17.6) | 12, 16 |
| " " | 290 | 279 | Mixed cancerous and noncancerous tissue | ♀ | (28.4) | 19 |
| " " | 210<br>290 | 196, 278 | Noncancerous liver adjacent to hepatoma | ♀, ♂ | (30.5) | 79, 46 |

ing *p*-dimethylaminoazobenzene. Almost all the human and rat cancers contained about the same amount of pantothenic acid as did noncancerous spleen, lung, and skeletal muscle, which are decidedly poorer in this factor than liver, heart, kidney, and brain. The mouse tumors studied, which included methylcholanthrene-induced sarcomas and transplanted adenocarcinomas, were somewhat richer, being on about the same level as the brain.

These results indicate that cancerous tissues probably do not have any greater need for pantothenic acid than do noncancerous tissues, but much more work is necessary before final conclusions can be drawn.

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# Possible Types of Mammary Gland Tumors in Mice*

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(Received for publication May 25, 1942)

Though the incidence may be low, no inbred strain of mice used for experimental studies on mammary cancer has been found to be entirely free from these tumors over a period of years. Other stocks have a high incidence of mammary carcinoma in virgin and/or breeding females. These differences may be a result of variations in:

(a) The inherited susceptibility or resistance of the mammary tissues to the development of spontaneous carcinoma.

(b) The amount of estrogenic stimulation of the mammary glands.

(c) The kind of milk influence obtained while nursing (7).

It has been assumed that mice of strains with a low incidence of mammary tumors are not susceptible to the spontaneous development of this type of neoplasm. A review of the literature (16) demonstrates, however, that the incidence may be low because animals have:

(a) An active milk influence and are not susceptible.

(b) An inactive milk influence and are not susceptible.

(c) An inactive milk influence and are susceptible.

Tumors which develop in susceptible or nonsusceptible mammary tissues may have the same histological structure, but as the inherited characteristics of the mammary glands are not identical their growths may, perhaps, result from different causes. How may these differences be demonstrated?

By observing the incidence of mammary tumors in the progeny of cancerous (and some noncancerous) mothers, it is possible to differentiate the types of mammary neoplasms arising in mice of inbred strains. In making these comparisons only those data obtained from animals subjected to normal breeding will be considered. The probable roles played by forced breeding and the administration of estrogenic hormones have been presented (16).

In an inbred strain of mice with a high incidence of mammary cancer, Strong (20) showed that there was no apparent difference between an animal which developed a tumor and one which did not. The progeny of noncancerous animals had a high tumor incidence. These observations have been confirmed on other high tumor strains (5, 13).

In the milk of females from high cancer stocks there is an active milk influence which they transfer while nursing; the influence in the milk of females from low cancer strains is usually inactive in the etiology of mammary cancer (4). This active milk influence may arise in mice which have not been in contact with animals having a high incidence of mammary tumors (11, 16) and so it would be possible for mice with a low incidence of mammary tumors to transfer the active milk influence. If these mice were used to nurse animals which had inherited the susceptibility for spontaneous mammary cancer, a high incidence of tumors would be found in these susceptible mice (10).

When the young born to females from high cancer strains of mice were given soon after birth to foster mothers of low cancer strains with the inactive milk influence, the incidence of mammary tumors in these fostered mice was very low. A few of the fostered mice which remained with their maternal parents of the high cancer stock for not longer than 24 hours before they were fostered developed mammary tumors. Many of their progeny and descendants did, also, but the incidence in these mice was not so high as in the controls (unfostered mice of the high cancer stock). A few fostered females without cancer, usually members of litters having one or more females with cancer, also had offspring that developed cancer but the incidence was not so high as in the progeny of the cancerous fostered mothers. Few of the progeny of the fostered mice that remained free of cancer developed mammary tumors. If any of the descendants of these fostered noncancerous mice developed mammary tumors their progeny had a low incidence, as did the progeny of their noncancerous litter mates (8). However, one female gave rise to a line with a high incidence (11).

If the young born to fostered noncancerous females or their descendants were not permitted to nurse their maternal parents, but were given to females of a high cancer stock with an active milk influence, a high

* This investigation was aided by grants from the National Cancer Institute and The Jane Coffin Childs Memorial Fund for Medical Research.

incidence of mammary tumors resulted (8). Thus foster nursing of mice from high cancer stocks does not change the inherited susceptibility of the fostered mice and their progeny to the development of spontaneous mammary tumors (11, 13, 14).

It is possible for a line (Ax) of mice descended from a fostered female of a high cancer stock (A) and having a low incidence of mammary tumors (4.5 per cent) to change to a high cancer (AxT) strain (96 per cent) without contact (nursing, etc.) with mice of a high cancer stock (11, 13). Also, females of the AxT strain which were free of cancer bore progeny of which a high percentage developed mammary tumors. Whereas the mice of the fostered Ax stock were shown to have an inactive milk influence, the mice of the AxT strain had an active milk influence (9, 13). They reacted in the same way as mice of the control or unfostered A stock.

Breeding females of the C57 black stock (17) and their hybrids, having and nursing maternal parents from this strain, had a low incidence of mammary tumors (6, 15). The hybrids and backcross generation animals gave evidence that mice of the C57 black stock were not susceptible to spontaneous mammary tumors (15).

Mice of the C57 black stock nursed by females of the high cancer C3H stock had an incidence of 10.3 per cent (1); when nursed by females of the high cancer A stock the *fostered* mice and their *progeny* had an incidence of 10.6 per cent (10).[1] The progeny of the fostered cancerous mice of the C57 stock had the same incidence of mammary carcinoma as did the progeny of the fostered noncancerous females. When these nonsusceptible fostered mice of the C57 stock were used to nurse mice which were susceptible to mammary tumors, the susceptible animals had a high incidence (10).

Andervont (2) found that breeding females of the C stock have an incidence of 2 per cent. A high incidence was observed in mice of the C stock fostered by females of the high cancer C3H stock (3) and also among the progeny of the fostered mice which died without having developed mammary cancer (personal communication).

## DISCUSSION

By observations on the incidence of mammary tumors in the progeny of cancerous and noncancerous mothers at least two types of tumors may be distinguished. These may be termed the inherited and noninherited types. It is possible that the inciting influences responsible for the production of these tumors are not identical (7).

[1] Fostered females of the C57 black stock when kept as virgins had the same incidence as the breeding females (21, 18).

Before mammary carcinoma can arise the gland must undergo development from stimulation of the estrogenic hormones. The amount of hormone produced in virgin females of different strains of mice is probably determined by intrinsic factors (19) and is not causally related to the incidence of mammary cancer. In breeding females, on the other hand, an amount of hormone is assured which is adequate to produce growth of the mammary tissue and accordingly to permit the cancerous change to occur when the other etiological influences are active. Thus the difference between the inherited and the noninherited type of mammary tumor in breeding females must be a result of variations in the character and concentration of the mammary tumor-milk influence and/or the intrinsic susceptibility or resistance of the mammary glands.

Mice of strains with a low incidence of mammary tumors may be susceptible (2) or nonsusceptible (6, 13, 15, 17) to spontaneous tumors.

If mice of susceptible strains are nursed by females with the active milk influence, a high incidence of mammary tumors will result in the progeny of these fostered mice whether they die with cancer or without. Under these conditions the growths would be termed "inherited tumors," whereas those arising in unfostered animals with an inactive milk influence would belong to the "noninherited" group.

Mammary tumors appearing in unfostered (inactive milk influence) and fostered (active milk influence) mice of nonsusceptible strains would be noninherited growths, as cancerous and noncancerous females would have progeny with a low incidence. While the active milk influence obtained from females of high cancer strains is able to overcome the threshold of nonsusceptibility of the mammary glands in only a few of the fostered animals, if the fostered mice are used to nurse mice susceptible to the development of spontaneous tumors a high incidence will result in these susceptible mice (10).

High cancer strains of mice may become low cancer strains by foster nursing to females of low cancer stocks having an inactive milk influence (4). This does not change the inherited susceptibility of the fostered mice and their progeny to the development of spontaneous tumors (14).

If the offspring of females of high cancer stocks are transferred before they have received any of the active milk influence from their potentially cancerous mothers, they and their progeny will have a low incidence and the tumors which develop will usually be not inherited. If the fostered mice obtain a small amount of active milk influence from their maternal parents before they are fostered they may develop cancer and bear cancerous progeny. The incidence of

cancer among the progeny will not be as high as that in the unfostered stock (8). In confirmation of recent observations (13, 15), the incidence was higher for those born in successive litters, which indicates that the concentration of milk influence increased with the age of the mothers and that the incidence of mammary tumors and the average tumor age may be partly determined by the amount of active milk influence received (9).

Inherited mammary tumors will result if the progeny of these fostered mice receive the active milk influence as the result of nursing (8). This influence may arise without contact with mice of high cancer strains (11). The amount of the active influence may be greater in the milk of females of some high cancer strains (18).

Thus there have been recognized three inciting influences in the production of spontaneous mammary tumors in mice. Since these will not arise in undeveloped mammary glands, the estrogenic stimulus must be considered as the primary inciting influence. While the estrogens will elicit the growth of mammary tissue, the architecture of the glands apparently is determined by the type of milk influence received while nursing (21, 19) and by intrinsic factors (21).

The feeding or injection of material containing the second influence, an active milk influence, produced few tumors when given to breeding females which had received inactive milk influence while nursing (Duran-Reynals and Bittner, unpublished). This suggests that if the architecture of the mammary tissue is established on the basis of an inactive milk influence, the addition of an active milk influence is unable so to alter this arrangement as to result in the formation of tumors.

The third influence is the genetic susceptibility or nonsusceptibility of the mammary tissue to the development of spontaneous mammary tumors (6, 7, 15). This is an inherited character, and genetic susceptibility may be transmitted by males and females of a susceptible stock.

The inherited type of mammary tumors develops in animals having all the active inciting influences. Noninherited mammary tumors arise in mice which receive the inactive milk influence and are susceptible or nonsusceptible, and in animals which have the active milk influence and are not susceptible to the development of mammary cancer.

It was decided several years ago to use the term "influence" (4) for the active principle in the milk of high cancer strains which plays an important role in the development of mammary cancer, until the nature of this agent had been determined. It has also been called the mammary tumor inciter (MTI) (22) and the cancer-inciting influence (2).

As it appears (7–16) that there must be at least three "inciting influences" active in the development of the inherited type of spontaneous mammary tumors in mice, it is improbable that any single influence can be considered as "the mammary tumor inciter." If one or more of the influences are inactive, a low incidence of mammary tumors usually results.

On the basis of the data available at the present time it is impossible to state if any one influence should be considered as the active mammary tumor inciter. The initial stimulus to the development of mammary cancer in mice takes place when the mammary glands start to develop as the result of estrogenic stimulation. This might be termed a "preparatory influence" in the development of mammary tumors. If it be so, the amount of estrogenic stimulation may determine the incidence and average age at which tumors will appear, a small amount resulting in a low incidence and a late tumor age and a large amount in a high incidence and an early tumor age (16). Furthermore, the amount of estrogenic stimulation produced in virgin females of various strains is probably the result of intrinsic differences and is not causally related to the incidence of mammary tumors (19).

The architecture of a precancerous gland is determined by the active milk influence and intrinsic factors (21, 19), but for a high incidence the active milk influence must be present when the glands are in the initial stages of development. As the estrogens must prepare the mammary tissues to become cancerous, so must the milk influence, and probably also the inherited susceptibility. Thus, all the active influences may be called preparatory influences, but as they are complementary influences they may be considered as mammary tumor inciters.

If the various influences or inciters are to be differentiated and given symbols, a distinction should be made between these various agents or factors. The active influence (stimulus, agent, factor, or principle?) in the milk from females of high cancer strains might be called the mammary tumor milk inciter (MTMI); the inherited factor (or factors) needed for susceptibility, the mammary tumor inherited inciter (MTII) or the mammary tumor susceptibility inciter (MTSI); and the hormonal influence, the mammary tumor estrogenic inciter (MTEI).

The MTMI would be a noninherited influence or inciter, the MTII or MTSI an inherited inciter, and the MTEI an intrinsic inciter in virgin females which may be supplemented by increased amounts resulting from extrinsic influences (breeding and injection).

The only purpose of these symbols would be to simplify reference to the various influences responsible for the development of mammary tumors. If they should be used in an attempt to express the concentra-

tion or the amount of stimulation of some influences resulting from intrinsic and/or extrinsic causes, only confusion on the part of the reader would be the result.

## SUMMARY

Observations on the incidence of mammary tumors in the progeny of cancerous and noncancerous mice indicate that there may be at least two types of mammary tumors—inherited and noninherited.

The inherited type develops in mice which receive active milk influence and are susceptible to the development of spontaneous growth.

Noninherited mammary tumors arise in animals which receive active milk influence and are nonsusceptible, or which receive inactive milk influence and are either susceptible or nonsusceptible to spontaneous tumors.

There are probably three mammary tumor inciters—the mammary tumor milk inciter (extrinsic); the mammary tumor estrogenic inciter (intrinsic and extrinsic); and the mammary tumor inherited inciter (intrinsic).

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# The Morphology and Development of Testicular Tumors in Mice of the A Strain Receiving Estrogens*

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(Received for publication June 6, 1942)

The administration of estrogens over periods of several weeks has been followed by extensive hypertrophy of the glandular interstitial tissue of the testis in mice of the Strong A strain (7) as well as in stock mice (4, 5). More prolonged treatment with estrogens has resulted in the appearance of testicular tumors in mice of the A strain (2, 15) and of the albino C strain (28). The present paper has as its objective a detailed description of the development and characteristics of testicular tumors in estrogen-treated mice of the A strain.

## EXPERIMENTAL

A total of 111 males of the A strain of Strong (31) was used in this experiment. Nine received 16.6 μgm. of estradiol benzoate weekly, 40 received 50 μgm. of estradiol benzoate weekly, 47 received 0.25 mgm. of stilbestrol weekly, and 15 were given 50 μgm. of estradiol benzoate and 1.25 mgm. of testosterone propionate weekly. Each of these substances was dissolved in sesame oil in such concentration that the weekly dose was contained in 0.05 cc. of oil. At the beginning of treatment the mice were 30 to 45 days of age, and the injections were continued until death occurred or appeared imminent. In 4 animals receiving 50 μgm. of estradiol benzoate treatment was discontinued after 6 months, and these animals were autopsied from 2 to 5 months later. Bouin's fluid served as the fixative, and the sections of the various organs were stained with Ehrlich's hematoxylin and triosin. The diet in all cases consisted of Purina fox chow, and water was available at all times.

None of the animals developed a testicular tumor after less than 8 months of treatment. The number of animals surviving this length of treatment was considerably less than the original 111, primarily because of changes associated with the treatment. For example, mammary tumors appeared in 50 per cent of the treated animals. Usually the mammary tumors were removed, although the mortality at operation was unfortunately fairly high. In many instances the treated animals exhibited the greatly distended bladders frequently seen after treatment with estrogens and the associated hydroureter and hydronephrosis, occasionally of sufficient severity to prove fatal. Many of the older animals had primary lung tumors and there was at least one instance of leukemia, although the treatment probably had no influence in producing either condition. The bones of the treated animals were affected by the injected steroids as described by Gardner and Pfeiffer (10, 11); in the animals receiving estrogen alone the long bones showed endosteal hyperossification and the pubic symphyses were replaced by a ligament, while these changes were less pronounced in the animals receiving both estrogen and androgen. The adrenal glands of most of the animals exhibited to various degrees the brown degeneration of Cramer (6), and in the older animals subcapsular hyperplasia was common. The hypophyses were examined in the first 40 mice at postmortem examination and in many instances were studied microscopically. No unusual feature was found. In view of this observation, the hypophyses were thereafter examined only occasionally. In one of these animals, however, a pituitary adenoma weighing 43 mgm. was present. The possibility exists that other animals also exhibited hypophysial change, but such a change was presumably quite rare.

Of the original 96 mice receiving estrogen alone, 56 survived 8 months' treatment. Of these 56 animals, 39 developed testicular tumors. In 18 animals both testes were tumorous, while in 21 the tumor was uni-

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, the Fluid Research Fund of Yale University School of Medicine, The Rockefeller Foundation, and the National Research Council, Committee for Research in Problems of Sex. The estradiol benzoate and testosterone propionate were furnished by the Schering Corporation through the courtesy of Dr. E. Schwenk, and the stilbestrol was supplied by E. R. Squibb and Sons through the kindness of Dr. J. A. Morrell. We should also like to express our gratitude to Drs. Edgar Allen, W. U. Gardner, and G. M. Smith for their generous advice and encouragement.

lateral. The incidence of the tumors as well as the survival of the animals is shown in Table I. These data indicate that stilbestrol is somewhat less toxic than estradiol benzoate and decidedly more effective in inducing the appearance of testicular tumors.

### Morphology of the Testicular Tumors

The testes classified as tumors weighed between 300 mgm. and 1.03 gm. (The weight of the testis of adult members of this strain rarely exceeds 150 mgm.). In the gross these testes were yellow-brown to dark red in color, rounded rather than oval, and typically exhibited considerable vascular congestion, especially superficially. In many the surface of the enlarged testis was smooth, while others had a distinctly nodular appearance.

Microscopically the tumorous testes were composed almost entirely of a single large mass or nodule of interstitial cells of Leydig (Fig. 18), surrounded peripherally by a narrow, sometimes discontinuous zone containing seminiferous tubules in various stages of atrophy. In a few instances enormously distended blood vessels were present throughout the testis. Small focal areas of necrosis were often present in the nodule, and in 4 instances the whole of a large nodule was necrotic.

Table I: Testicular Tumors in Mice of the A Strain Receiving Estrogens

| Treatment | Number of animals | Number living after 8 months | Number with testicular tumors | Number with nodes involved | Number showing action of androgen |
|---|---|---|---|---|---|
| 16.6 μgm. estradiol benzoate weekly | 9 | 7 | 3 | 2 | 3 |
| 50 μgm. estradiol benzoate weekly | 40 | 17 | 7 | 5 | 7 |
| 0.25 mgm. stilbestrol weekly | 47 | 32 | 29 | 22 | 25 |
| Total | 96 | 56 | 39 | 29 | 35 |

The interstitial cells comprising the nodules were of 3 distinct types. The first type was 2 or 3 times as large as the Leydig cell of the untreated mouse (Fig. 2). Its cytoplasm was highly vacuolated and its granules were very large and stained a vivid crimson with eosin. The nuclei were somewhat enlarged and often contained eosinophilic granules. Mitoses were very rarely encountered in these cells, and fibroblasts were distributed among the Leydig cells. A second variety of interstitial cell (Fig. 3) resembled the normal Leydig cell of the mouse in size and in being much less vacuolated than the large cell. This type of cell stained a dull red with eosin and an occasional mitosis was found. A third type (Figs. 4, 5) was never more than one-half as large as the normal Leydig cell and its cytoplasm was more or less basophilic. These cells were typically hyperchromatic and exhibited many, and often apparently abnormal, mitoses.

Two of the tumors were made up of the first type of cell, 10 of the second, and one was composed entirely of the small, third type of cell. The remainder were mixed; 8 contained all 3 types of cells, 5 contained medium and small cells, and the remainder contained the large and medium sized cells. In the mixed tumors the different types of cells were usually not indiscriminately mixed; instead, each variety was more or less localized in nests of various sizes (Fig. 4). In 5 of the tumors the large cells were organized into cords covered by endothelium. The medium and small cells have not been observed to form such cords.

Tubules were not at all numerous in these testes and, when present, they were in the terminal phases of atrophy. They were, with a few isolated exceptions, located peripherally to the tumorous nodule (Figs. 17, 18) and the intertubular spaces contained two varieties of cells. One was much like the large cells of the nodules, except that its cytoplasmic granules were smaller and had less affinity for eosin. These cells did not appear to be vacuolated, and were probably undergoing degeneration. The second variety of peripheral intertubular cell had yellow-brown cytoplasm and one or many small, often pyknotic, nuclei. These cells varied enormously in size, ranging from cells slightly larger than lymphocytes to multinucleated masses one-half as large as a tubule in transverse section. Connective tissue fibers were also abundant in the peripheral zone containing the tubules.

### Invasiveness

The nodules were typically circumscribed, although in 7 instances the cells of the nodule showed here and there some infiltration of the surrounding intertubular spaces. When the peripheral zone of tubules was discontinuous the nodule abutted on the tunica albuginea in places. In 3 such growths the tunica albuginea was invaded by the tumor cells (Fig. 6). In 4 others a similar invasion seemed possible; but here this appearance could have resulted from the crowding together of the basement membranes of completely atrophied tubules and their captured peripheral cells in such a way as to simulate the deeper portion of the tunica albuginea.

The lumbar and renal lymph nodes and, less often, the mediastinal nodes in animals bearing the tumors

were enlarged to various degrees and tan in color. In one animal a lumbar node weighed 52 mgm. and a renal node 102 mgm. These nodes were invaded in 29 of the tumor-bearing animals (Fig. 7). In 5 animals only a few tumor cells were present, while in 10 the metastatic involvement of these nodes was so extensive that their lymphoid character was almost or completely lost.

In 3 growths composed of small and intermediate cells the lumina of several venules or sinusoids contained tumor cells. In no instance, however, have metastases other than lymphatic been identified. In several animals the fat of the spermatic cord was dark red or yellow in color, and it gross appearance suggested metastasis, but upon microscopic section the area in question seemed to be composed only of fat corpuscles in different stages of maturity.

### Transplantation

Attempts to transplant 5 of the tumors were made, with 10 to 16 hosts on each occasion. A bit of the tumor or of an invaded lymph node was grafted subcutaneously into the axilla of male and female hosts or into the testis. As found by Bonser and Robson (2) and by Shimkin and his coworkers (28), the grafts failed to grow in untreated hosts. When, on the other hand, the hosts were given weekly injections of the estrogen received by the donor animal, the tumor or invaded node of 3 of the donors grew when transplanted (Fig. 8). In each instance 5 to 8 host animals were treated, and each of the 3 tumors which were successfully transplanted grew in 2 of them. The initial rate of growth of the transplants in the treated hosts was extremely slow; but after 6 months the growth accelerated and the grafts eventually attained a size greater than that of the original tumor. One of the grafts was transplanted into a second generation of hosts, and here again grew only if estrogen were injected. The tumors which failed to graft proved to be composed of the large, highly vacuolated cell of type I. The other 3 tumors contained predominantly medium sized cells of type II. No attempts to transplant tumors composed primarily of the small, hyperchromatic cells (type III) have thus far been made.

### Production of Androgen

In 35 of the animals bearing testicular tumors the seminal vesicles, prostate glands, and coagulating glands closely resembled, grossly and microscopically, these structures in intact, untreated adult males, indicating that they were under the stimulation of amounts of androgen sufficient not only to maintain their normal structure but to offset with various degrees of completeness the effects of the injected estrogen on these organs. The section of a seminal vesicle shown in Fig. 9 is typical of those of the tumor-bearing animals.

The accessory glands of reproduction showed no signs of stimulation by androgen unless vast numbers of interstitial cells were present in the testis, indicating that the tumor cells were the source of the androgen. The fact that only estrogen effects were observed in these organs after months of treatment when no tumor formed suggests that the androgen most likely did not arise from some extratesticular source under the influence of the injected estrogen.

### Development of the Tumors

*Early effects of estrogen.*—The earliest change in the testis following the start of administration of estrogens was an apparent stimulation of the Leydig cells involving increase in their size and vacuolation without notable change in their number or any effect upon the tubules. This change was seen after not less than 50 days of treatment, and the duration of this phase of apparent stimulation was highly variable, ranging from 3 to 6 months or even longer, during which time these cells progressively increased in size (Fig. 10) until they were 4 or 5 times as large as the normal cell. In these greatly enlarged cells the cytoplasm contained widely separated and extremely large, discrete, poorly stained granules not apparently separated by vacuoles or by smaller granules. As these cells aged the individual granules became somewhat smaller and showed still less affinity for eosin, and the nuclei showed early pyknosis. Concurrently with these changes in the enlarged Leydig cells, a new cell type appeared. These cells were usually quite large with small, often pyknotic nuclei and a brown cytoplasm. The brown cells made their appearance simultaneously with the macroscopic acquisition of the yellow-brown color of the testis.

*Regression.*—Several observations suggest that the brown cells are phagocytic. They were most numerous in areas of disintegration (without much necrosis) of Leydig cells, and were usually present in broken, atrophic tubules. They contained a single nucleus when their size was small, and were usually multinucleated when their size was large (Fig. 11). In several instances they had engulfed Leydig cells (Fig. 13). To investigate further the possible phagocytic activity of the brown cells, India ink was injected directly into the testis in mice which had received estrogen for a sufficient period to assure the presence of these cells. The India ink was taken up selectively by the brown cells (Fig. 14) and by no other testicular element. The brown cells in the renal and lumbar lymph nodes

of these animals phagocytized the ink particles in the same manner as the cells of the testis. No unequivocal intermediate stages were seen which would indicate a transformation of Leydig cells into brown cells.

By the time the testis had attained the intertubular morphology just described, the tubules, which were still uniformly distributed through the testis, showed signs of atrophy in scattered areas. The gross size of the testis, which had been maintained essentially unchanged, now began to decrease. This diminution in size was characterized microscopically by a widespread disappearance of Leydig cells (Fig. 11) and by partial atrophy of the tubules. The loss of interstitial cells appeared to be to a considerable extent the result of their phagocytosis by the brown cells. The removal of the Leydig cells was quite complete, and upon "emigration" of the brown cells the intertubular spaces contained only mesenchymal elements, fibroblasts, and connective tissue fibers (Fig. 12). During the terminal phase of the removal of Leydig cells, plasma cells could be identified in the intertubular spaces. Since the pace of events was, of course, never precisely the same in the entire testis, no testis revealed cleared intertubular spaces throughout. That it is a definite and constant occurrence, however, seems unmistakable.

*Formation of nodules.*—Subsequent to the removal of the Leydig cells a new generation of interstitial cells formed, the cells of origin being mesenchymal or fibroblastic as indicated by the intermediate stages. The formation of new Leydig cells occurred throughout the testis but was more rapid in one or more localized areas, with the result that one or more nodules of Leydig cells formed (Fig. 15). The growth of the nodule pressed the surrounding tubules further and further apart, ultimately to the periphery of the testis, and it was in the stage of nodular growth that wholesale tubular atrophy became apparent. Throughout the growth of the nodule the central cells were somewhat smaller than the peripheral cells, which were admixed with brown cells. Further growth of the nodule led to its comprising the bulk of the testis, while the tubules, old enlarged Leydig cells, and brown cells surrounded it peripherally. Obviously the displacement of the stroma and of the basement membranes of atrophied tubules would serve to circumscribe the nodules. The growth of the nodules accounted entirely for the abnormal increase in size of the testis of the treated animals, and the signs of androgenic activity appeared only when the nodule or nodules of Leydig cells had attained significant size. Moreover, invasion of the lymphatics occurred only when large nodules were present. Four stages of the growth of a nodule are shown in Figs. 15 to 18.

*Generations*[1] *of tumor cells.*—There appeared to be 3 distinct generations of cells in the nodules. The first was the enlarged, highly vacuolated cell whose origin from mesenchymal or fibroblastic elements has just been described and which contains large, strongly eosinophilic cytoplasmic granules (Fig. 2). This was the only Leydig cell in the small nodules, and occasionally was the only cell type in a large tumor. With further treatment a second generation of cells appeared, and partially or completely replaced the cells of the first generation. The second generation of cells was the medium-sized cell (Fig. 3) which resembled the normal Leydig cell. Upon still longer treatment a third generation of small, basophilic, hyperchromatic cells appeared. These cells were first seen in several localized areas in the nodules of second generation cells (Fig. 4) and in one instance came to comprise the entire tumor (Fig. 5).

The duration of treatment required to produce any given generation of tumor cells varied considerably, and on one occasion the growth was composed primarily of first generation cells even after 15 months of treatment. The sequence of events seems, however, to have been the same in all the animals, although the speed may have varied.

The foregoing description of the sequence of morphological events in the transformation of the normal testis into an interstitial cell tumor is based upon all the testes examined. In many instances the two testes from a single animal exhibited widely different degrees of modification; and while the tumorous testis offered no clue as to the sequence of events, the less advanced testis clearly indicated intermediate stages. The several intermediate steps are summarized in Fig. 1. To indicate temporal relations this chart is based solely upon the condition of the more modified of the two testes. For this reason the individual early stages are not represented as exhibiting what is very probably the correct duration of their existence. The fact that very few animals were autopsied after less than 6 months of treatment also contributes to the incompleteness of this portion of the chart.

The cells of the different generations appeared to vary somewhat in their properties. The androgenic effects were manifest during the phase of the first generation cells, becoming more noticeable as these cells increased in number. The androgenic effects appeared to decline somewhat when the second and third generations of cells replaced the first, indicating a higher secretory activity of the first generation cells. Invasions of the tunica albuginea and infiltration of the lymphatics have occurred only when the tumor

[1] The term "generations" is used here in the sense of successive crops of cells from the same parent stock, and does not denote filial descent.

was composed of cells of either or both the second and third generations. The tumors which failed to grow when transplanted into hosts treated with estrogen were composed of first generation cells, while the 3 composed of cells of the second generation were transplanted successfully into treated hosts. The autonomy of cells of the third generation has not as yet been tested. Finally, endothelium-covered cords of cells of the first generation have developed in 5 animals, while cells of the later generations have not been observed to form such cords.

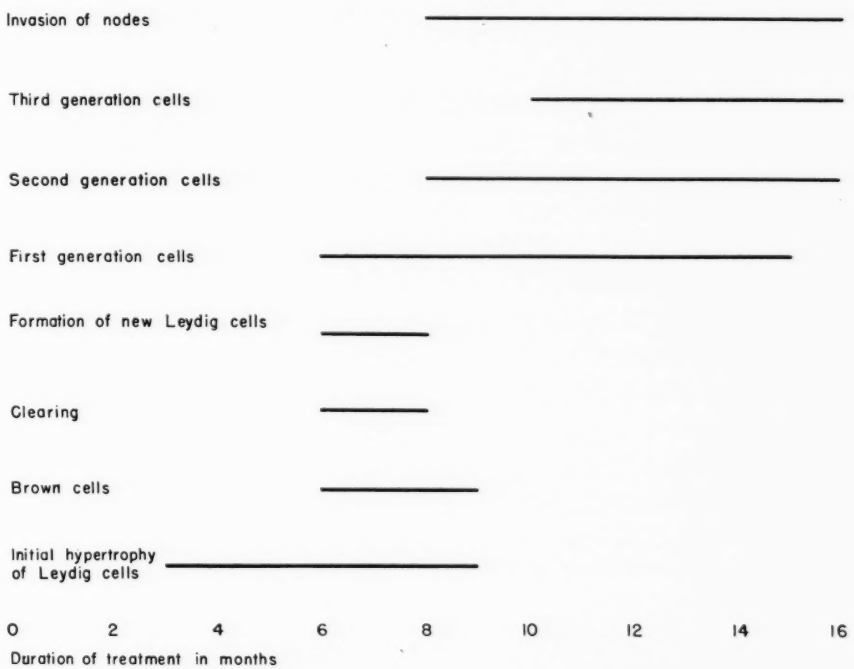



FIG. 1.—The apparent sequence of events in the transformation of the normal testis into a Leydig cell tumor and the duration of each phase in estrogen-treated mice. This chart is based solely upon the condition of the more modified of the two testes and a line indicates that animals autopsied throughout the range of treatment shown exhibited that particular phase. Two or more phases may be present in a single testis. Thus after 12 months of treatment, for example, 1, 2, or all 3 generations of tumor cells may be present; and in any one animal the nodes may or may not be involved.

*Reversion.*—When treatment was discontinued after 6 months, that is, when the testis was in the phase of Leydig cell disintegration or early new formation, the testes reverted to an essentially normal histology and several intermediate stages in the reversion were obtained. Two months after treatment had been discontinued the histology of the testes remained what it is typically after 6 months of treatment. A month later the brown cells were much less numerous, and the hypertrophied Leydig cells had nearly disappeared. Two months later both the brown cells and hypertrophied Leydig cells were entirely absent. Three months later, that is, 5 months after termination of injections, the testis had entirely recovered, with the tubules and intertubular tissue like those of an untreated mouse.

*Retardation by androgen.*—In the 15 animals receiving simultaneously both estradiol benzoate and testosterone propionate 2 testicular tumors developed, both after 14 months of treatment. The morphologic events in the testis culminating in the development of tumors were the same as in the animals receiving only estrogen, with the difference that the male hormone evidently exerted a considerable retarding influence. The tumors were histologically like those in the animals receiving only estrogen, and were composed of cells of the first and second generations. The lymph nodes of both animals were free of tumor cells.

## DISCUSSION

Interstitial cell tumors of the testis following treatment with estrogens have thus far been reported in two strains of mice, the A strain (2, 15) and the albino C strain (28). Several estrogens, estradiol benzoate at two dose levels, stilbestrol, and triphenylethylene, have proved effective in this respect. The incidence of the tumors appears to be somewhat variable. Of 23 males of the A strain receiving triphenylethylene, apparently 13 developed testicular tumors (2) after a minimum of 11 months' treatment. Of 62 surviving males of the albino C strain carrying pellets of stilbestrol-cholesterol, 13 developed testicular tumors, 6 months being the minimum effective period of treatment (28). In the present experiment the minimum effective period of treatment was 8 months, with 10

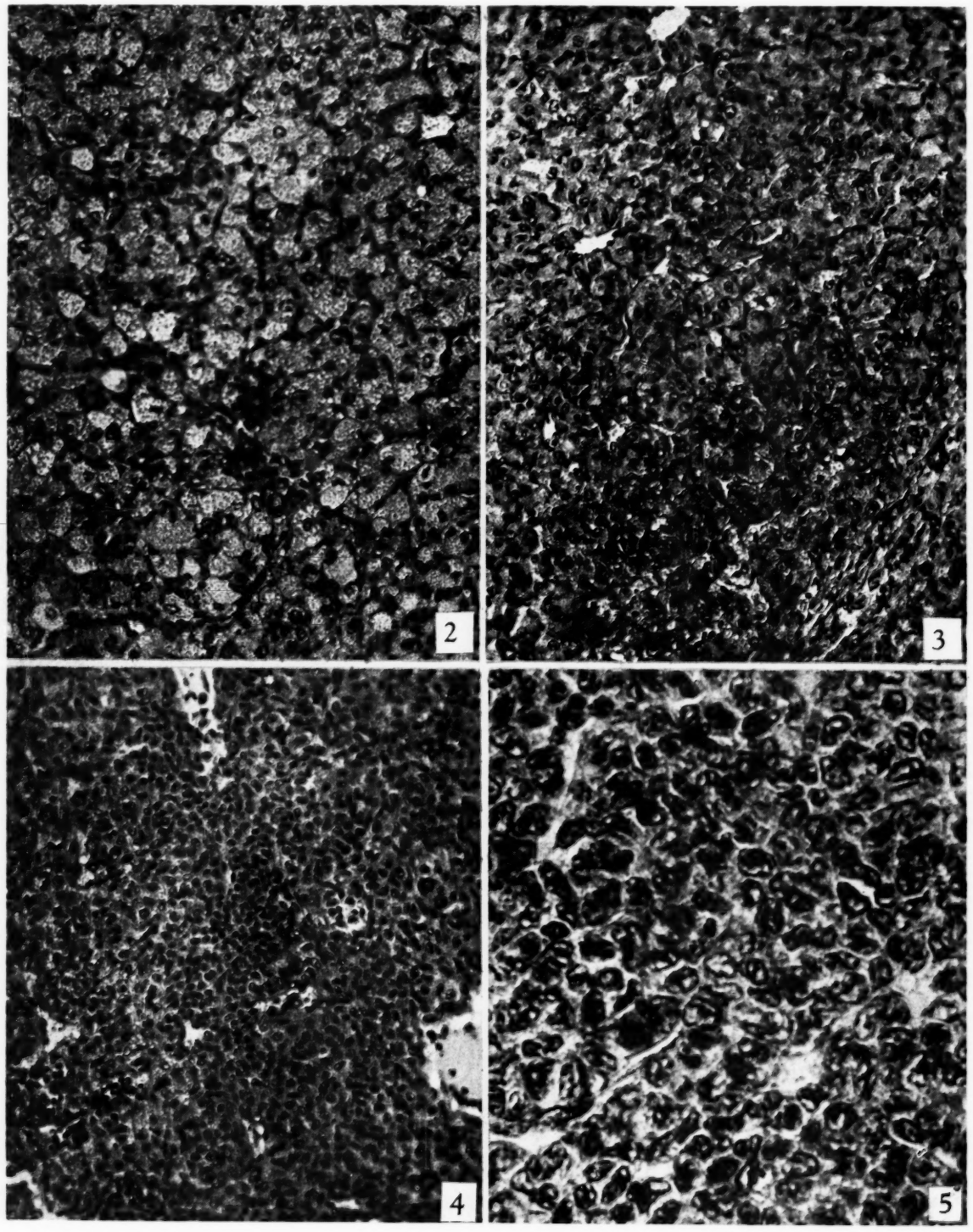



FIG. 2.—An area of large, highly vacuolated cells with large, vividly eosinophilic cytoplasmic granules (cells of the first generation) in a tumor composed of 3 types of interstitial cells. Numerous fibroblasts are present. The animal bearing this tumor had received 0.25 mgm. of stilbestrol weekly for 12½ months. Both testes were tumorous. Mag. × 175.

FIG. 3.—An area of a tumor composed entirely of medium sized Leydig cells (cells of the second generation). These cells are moderately vacuolated, have smaller granules, and stain a dull red with eosin. The animal had received stilbestrol for 8 months. The tumor was unilateral, and the lymph nodes were invaded. Mag. × 175.

FIG. 4.—A nest of small cells (third generation) in an area composed of medium-sized cells (second generation). The tumor was unilateral and composed of cells of all 3 types. The animal had been given stilbestrol for 12 months and was autopsied 1 month after cessation of treatment. The lymph nodes were invaded. Mag. × 175.

FIG. 5.—An area of a tumor composed entirely of small cells (third generation). These cells are hyperchromatic and show frequent mitoses. The animal had been given 0.25 mgm. stilbestrol weekly for 10 months. Mag. × 750.

of 24 surviving A males receiving estradiol benzoate and 29 of 32 surviving A males receiving stilbestrol developing testicular tumors. Shimkin and his associates (28) observed testicular tumors after 7 months

A comparison of the invasiveness of these tumors is impossible on the basis of the reported data. One apparent discrepancy does exist, however. Both Bonser and Robson (2) and Shimkin and his associates

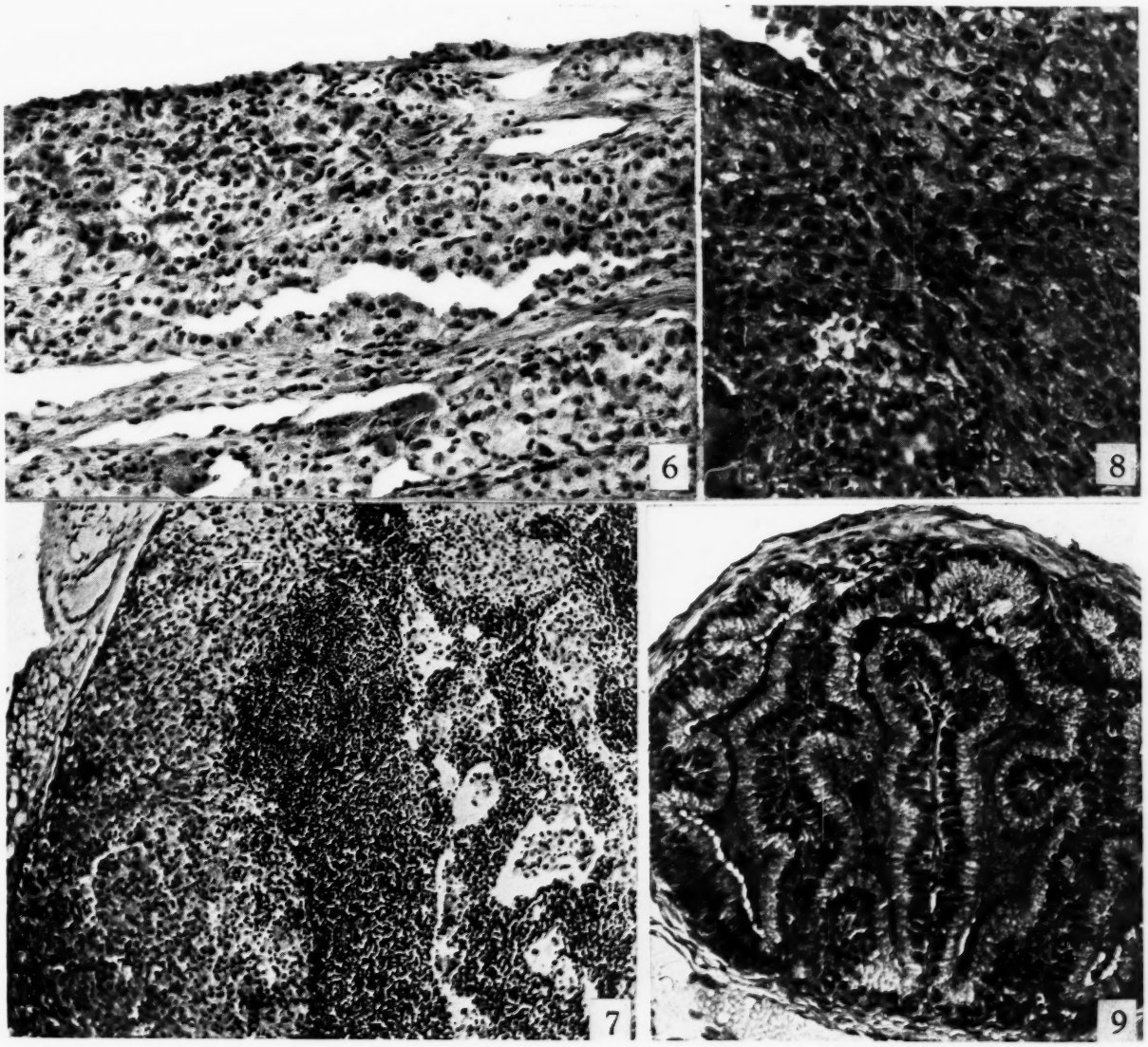


FIG. 6.—An area at the periphery of a tumor showing invasion of the tunica albuginea. This animal received 50 μgm. estradiol benzoate weekly for 8½ months. The tumor was unilateral and composed of medium sized cells. The lymph nodes were invaded. Mag. × 175.

FIG. 7.—A peripheral portion of a lumbar lymph node from the same animal, showing infiltration with tumor cells. Mag. × 85.

FIG. 8.—A portion of a transplant of the same tumor after 7 months' residence in an estrogen-treated host of the A strain. Mag. × 175.

FIG. 9.—A section of a seminal vesicle of a mouse bearing a unilateral testicular tumor. The tall epithelium and the presence of secretion in the lumen indicate the action of androgen. This animal had received 16.6 μgm. estradiol benzoate weekly for 15 months. Mag. × 85.

in 2 of 20 mice of the A strain carrying stilbestrol-cholesterol pellets. So far as can be judged, the tumors in all cases went through the same developmental phases and are alike morphologically. It is probable, therefore, that the various estrogens operate in much the same manner to lead to the development of testicular tumors.

(28) describe nodules of tumor cells in the perirenal fat or fascia immediately caudad to the left adrenal gland. Both groups of workers felt that this nodule did not represent a lymph node. In all the animals in the present study a lymph node was found in precisely this location. In this node all the intermediate stages between the presence of no tumor cells and the virtu-

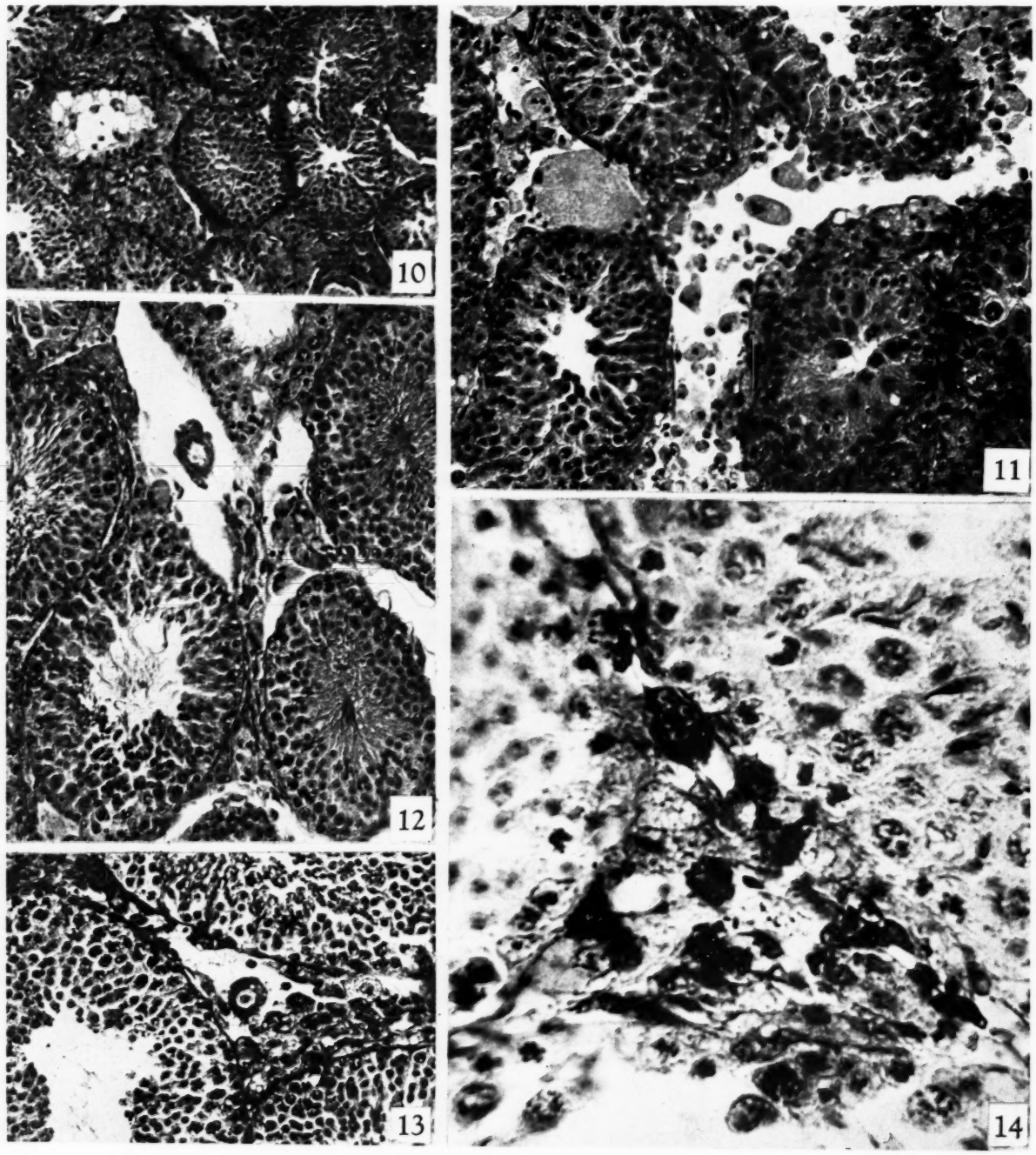



FIG. 10.—An area of a testis of a mouse after 6 months' treatment with 50 μgm. estradiol benzoate weekly showing the early effects of the treatment. Most of the tubules are fairly well preserved. The Leydig cells are enlarged and highly vacuolated. In the lower right corner a small group of brown cells may be seen. Mag. × 85.

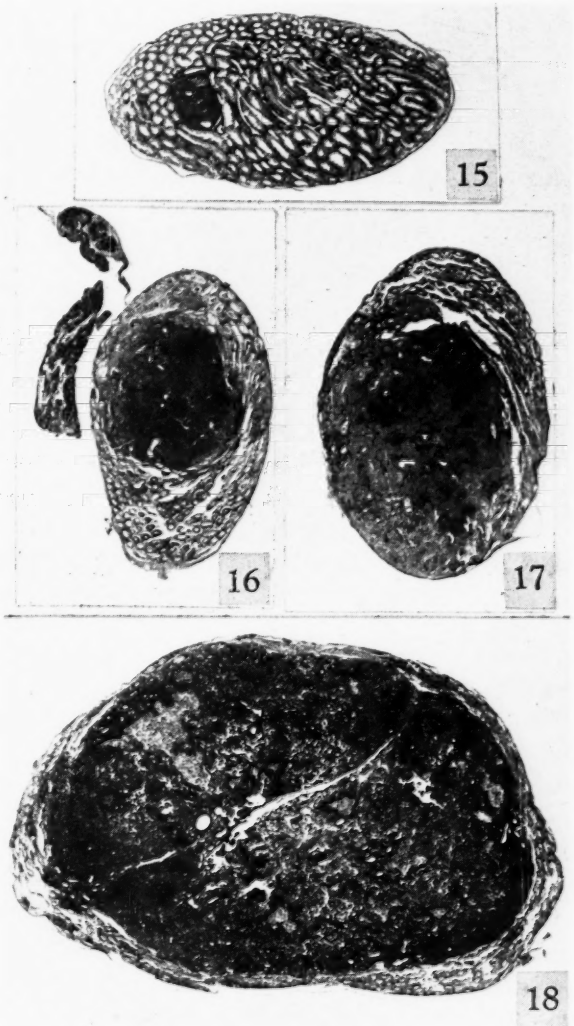
FIG. 11.—An area of a testis showing loss of Leydig cells (*cf.* Fig. 10) and the presence of brown cells of different sizes. This animal had been given 50 μgm. estradiol benzoate weekly for 9 months. Mag. × 175.

FIG. 12.—An area of a testis showing loss of Leydig cells and diminution in the number of brown cells. Formation of new Leydig cells occurs by transformation of mesenchymal or fibroblastic elements in areas such as that in the center of the field. This animal had received 50 μgm. estradiol benzoate weekly for 7 months. Mag. × 175.

FIG. 13.—A Leydig cell engulfed by a brown cell is shown in the center of the field. This testis is from an animal treated for 6 months with 0.25 mgm. stilbestrol weekly. Mag. × 175.

FIG. 14.—An area of the testis of a mouse treated with stilbestrol for 6 months and given a single intratesticular injection of India ink. The brown cells have phagocytized the ink particles while the Leydig cells are free of ink. Mag. × 700.

ally complete replacement of the lymphocytes by tumor cells have been seen. A lymph node was present on the left side more frequently than on the right, and the involvement was correspondingly more frequent on the left side. Indeed, the left renal node was invaded more often than the lumbar nodes. Similarly, when India ink was injected into the testes it accumulated more abundantly in the left renal node than in the other lymph nodes, suggesting that the largest testicular lymph channel drains into the left renal node.




FIGS. 15-18.—Stages in the growth of nodules of interstitial cells. The progressive atrophy and loss of tubules as they are displaced toward the periphery are shown. All figures, Mag. × 7.3.

FIG. 15. After 6 months' treatment with 0.25 mgm. stilbestrol weekly.

FIG. 16. After 9 months' treatment with 50 μgm. estradiol benzoate weekly.

FIG. 17. After 7 months' treatment with 0.25 mgm. stilbestrol weekly.

FIG. 18. After 10 months' treatment with 0.25 mgm. stilbestrol weekly.

The interstitial cell tumors of the testis obtained in estrogen-treated mice are apparently identical microscopically with those which have arisen spontaneously in a mouse (29), in dogs (3, 12, 18, 24, 33), in horses (24), and in man (3, 16, 17, 26, 27, 30, 32). In view of the similarity of the tumors its seems not unlikely that if the means by which injected estrogen leads to the formation of testicular tumors in mice were known, the factors responsible for the development of spontaneous interstitial cell tumors would likewise be indicated.

An original incentive for examining the carcinogenic powers of the estrogens (23) lay in the fact that these substances are growth stimulants acting primarily upon the female genital tissues (1). It was thought that long continued action of rather large amounts of these stimulants might lead ultimately to abnormal or even neoplastic growth; and the development of mammary (19) and cervical (9) carcinomas in estrogen-treated mice bore out the expectation. In the development of the uterine tumors in estrogen-treated mice, however, a period of uterine regression intervenes between the original stimulation and the appearance of the tumor (8). In the testis, in the present experiment, an initial stimulation of the interstitial cells was followed by more or less complete removal of these cells. Thus the initial response did not proceed progressively to the formation of a tumor. Instead, there supervened a phase of interrupted response followed by alteration in the type of response.

The identity of the brown cells which appear to be involved in the removal of the initially hypertrophied Leydig cells is uncertain. They bear some resemblance to the brown cells in the adrenal glands of these animals showing the brown degeneration of Cramer (6), although their refractility is different (28). The testicular brown cells appear to be identical with brown cells in the lymph nodes of old, treated and untreated mice of both sexes in the A strain; and the brown cells in both locations phagocytize ink particles in the same fashion. At the moment it seems reasonably certain that the brown cells in the testis are phagocytic, and it seems probable that they are macrophages, as has been suggested for the brown cells in the adrenal gland (20); but the possibility of their origin from Leydig cells has not been excluded.

To what extent the clearing of the intertubular spaces is the result of phagocytic activity of the brown cells is uncertain. That there is much of it seems probable, although plasma cells and possibly blood elements may assist in the process. At any rate, phagocytosis must account for a great deal of the clearing, and the clearing appears to be a constant phenomenon preceding the development of the tumor.

The distintegration of the Leydig cells in the treated mice conforms to the usual concept of gonad-hypo-

physis relationship (22) and may suggest hypophysial suppression. The hypertrophy preceding disintegration, however, recalls the action of injected estrogen in evoking release of luteinizing hormone (21), a substance which in the male is reported to elicit hypertrophy of Leydig cells (13). The accumulation of nests of Leydig cells in the testes of mice of the A strain receiving daily injections of pregnant mare serum over periods of several months (25) accords with the possibility that the effects of the estrogen are mediated by the hypophysis.

In the development of the normal Leydig cell two separate phenomena are recognizable (14). The first is the metamorphosis of mesenchymal or fibroblastic elements into small, polygonal, granular, epithelioid cells. The second is the transformation of the granular cell into a larger, highly vacuolated cell. The presence of the latter type of cell is accompanied by a high level of production of androgen, while the granular cell apparently has a low level of androgen production.

The administration of estrogen to males of the A strain was at first followed increasingly by the second phenomenon; that is, by growth and vacuolation with little or no metamorphosis of new cells. Ultimately the enlarged cells died, possibly of "exhaustion," and were removed. Then followed an increasingly rapid formation of new Leydig cells and their prompt excessive vacuolation and enlargement. These cells were the cells of the first generation. After a time the rate of formation came to exceed somewhat the rate of enlargement and vacuolation, with the result that cells of the second generation appeared. Finally, the act of formation so dominated that a generation of small, nonvacuolated cells (the third generation) appeared. This generation, much more than the earlier ones, has the power to increase itself by proliferation.

It is not known, of course, whether separate stimuli are responsible for the two distinct phenomena in the normal Leydig cell. It may be significant, however, that in old age, when interstitial cell tumors develop spontaneously in animals, the Leydig cells are predominantly granular.

The effects of the estrogen in the early months of treatment clearly are not irreversible, as shown by the recovery of the testes of the animals in which treatment was discontinued after 6 months. The failure of the testes of these animals to proceed to the formation of tumors, coupled with the fact that long continued treatment is required to produce the tumors, suggests that chronic treatment is required to evoke a physiological state which, in turn, results in massive formation of new Leydig cells. Such a supposition is in keeping with the failure of the grafts to grow in untreated hosts, and would account for the initially extremely slow and later rapid growth of the grafts in the treated hosts. A second possibility is an initial (direct?) effect upon the testis which alters its capacity to react to an altered animal physiology.

## SUMMARY

Twenty-four of 49 mice of the A strain receiving 16.6 or 50 μgm. of estradiol benzoate weekly, and 32 of 47 mice of the A strain receiving 0.25 mgm. of stilbestrol weekly, survived 8 months of treatment. In 10 of the former group and in 29 of the latter group unilateral or bilateral testicular tumors developed. No testicular tumors developed in mice treated less than 8 months.

The tumors consisted of a large nodule of interstitial cells of Leydig surrounded by a narrow, sometimes discontinuous zone of atrophic tubules. The tunica albuginea was invaded in 3 of the tumors. The renal, lumbar, and mediastinal lymph nodes were infiltrated by tumor cells to various degrees in 29 of the 39 tumor-bearing animals. No other metastases have been recognized, although tumor cells were seen in venules of the testis in 3 animals. The seminal vesicles and prostate glands of 35 of the tumor-bearing animals evinced the production of high levels of androgen by the tumors. Attempts to transplant 5 of the tumors to untreated hosts were unsuccessful. In mice that were given estrogen 3 of the tumors grew when transplanted.

The sequence of events in the testes culminating in the formation of tumors appears to be as follows: There is first an enlargement of the Leydig cells and the appearance of brown cells (possibly macrophages) in the intertubular spaces. Then follows a disappearance of the Leydig cells, apparently to a considerable extent through their phagocytosis by the brown cells. After the clearing of the intertubular spaces, new Leydig cells appear to arise by metamorphosis of mesenchymal or fibroblastic elements. The formation occurs more rapidly in localized areas, with the result that one or more nodules arise. Continued growth of the nodule accounts for the gross enlargement of the testis. There appear to be 3 generations of tumor cells: the first is large, vacuolated, and strongly eosinophilic, the second resembles the normal Leydig cell, and the third generation is a small, fairly basophilic, hyperchromatic cell with frequent mitoses. Tumors were composed of any one generation or of combinations of 2 or all 3 generations of cells.

When 1.25 mgm. of testosterone propionate was given concurrently with 50 μgm. of estradiol benzoate, 2 of 15 mice of the A strain developed testicular tumors. The androgen appeared to retard, but not to prevent, the changes in the testis.

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# The Relation of Diet to the Development of Gastric Lesions in the Rat

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(Received for publication June 3, 1942)

Cancer of the stomach in man is one of the most common of all tumors (34), but in animals it is a rare finding (63).

It has long been suspected that gastric cancer is caused by chronic irritation and unbalanced diets. Fibiger (18) in 1913 succeeded in producing lesions in the forestomach of rats, which he believed to be squamous cell carcinoma, by infesting them with the parasite *Gongylonema neoplasticum*. He thought that the tumors were elicited by the parasites, but, in a paper published in 1920 (20), emphasized the fact that the animals were kept on a diet consisting only of white bread and water. Passey (42) suggested that such a diet must have been deficient in vitamins, and on the basis of this assumption carried out an experiment in which he showed that vitamin A deficiency, in the absence of the *Gongylonema*, led to papillomatosis of the forestomach of the rat. Cramer (12), however, found that warts or papillomas might occur in the forestomach of old rats which had received an adequate amount of vitamins.

Stimulated by Fibiger's observations, many workers have made attempts to produce gastric cancer in different species of animals by feeding parasites, artificial and deficient diets, and chemicals known or suspected to be carcinogenic. The results obtained from these studies are summarized in Table I, which presents, though admittedly in uncritical form, all the published observations. It is hoped that this will be a convenient supplement to the critical review by Klein and Palmer (32, 33). Since the publication of this review Brunschwig and Rasmussen (5) have described ulceropapillomas in the stomach of rats fed a variety of unbalanced diets such as high fat (lard), high carbohydrate (rice), high protein (casein), with and without vitamins A, $B_1$, $B_2$, C, and D. The lesions did not appear to be the direct result of deprivation of the vitamins mentioned above, or caused by heated fats, but are referable to a certain degree of malnutrition or possibly to a deficiency of some vitamin as yet unidentified.

The most interesting and best established observations revealed by all these data are that the incidence of the gastric lesions is definitely related to dietary deficiency and that they are found generally in the rumen (forestomach).

Certain facts should be pointed out concerning these attempts to produce cancer of the stomach. First, rats have been used almost exclusively, since guinea pigs, mice, and rabbits do not develop gastric tumors. Therefore, any conclusions drawn must be restricted to the rat. Second, the tumors produced in the forestomach of rats have practically all been described as papilloma and only occasionally as carcinoma. In the glandular portion cancer is still more rare. Third, animals have often died long before the expiration of 14 months, the average induction period for both benign and malignant squamous cell tumors of the forestomach with methylcholanthrene. The time factor appears to be very important.

The object of the present study was to find whether or not a combination of an irritant and a deficient diet could produce squamous cell carcinoma in the forestomach, or adenocarcinoma in the glandular portion of the stomach, in rats.

## EXPERIMENTAL

In this investigation polished rice was selected for the production of gastric lesions in rats, because young rats fed on polished rice will develop ulceropapillomas in the forestomach in the course of 30 to 43 days (5). As a mechanical irritant kieselguhr was selected.

Young albino rats of the Sherman stock, from 30 to 75 days old and weighing from 60 to 125 gm., were segregated from stock animals (which were fed upon a regular diet of Purina laboratory chow) and placed on the mixture of polished rice, 95 per cent, and kieselguhr, 5 per cent. Unlimited water was allowed.

As a control a number of young rats were maintained upon polished rice alone. Others were kept on a diet of unpolished rice (brown rice) with or without carrot.

Feeding on the respective diets was continued until the death of the animals, at which time their stomachs were examined in the gross and microscopically.

The results (Table II) show clearly that the daily oral administration of kieselguhr had a definite stimulating effect upon the production of lesions in the forestomach. Of 21 rats in the kieselguhr group, 20, or 95 per cent, showed lesions (2 to 32). In the non-kieselguhr group, 29 out of 51 rats, or 57 per cent, had lesions. They appeared like umbilicated warts, measuring 2 to 4 mm. in diameter, and were elevated above the surface 1 to 2 mm. (Fig. 1). These small nodules had punctate hemorrhages in the center. They proved on microscopic examination to be epithelial hyperplasia and hyperkeratosis; there were no evidences of malignant change (Fig. 2). Occasionally ulceration of the epithelium was noted. The glandular portion was free from the hyperplastic lesions but quite frequently diffuse hemorrhages were observed.

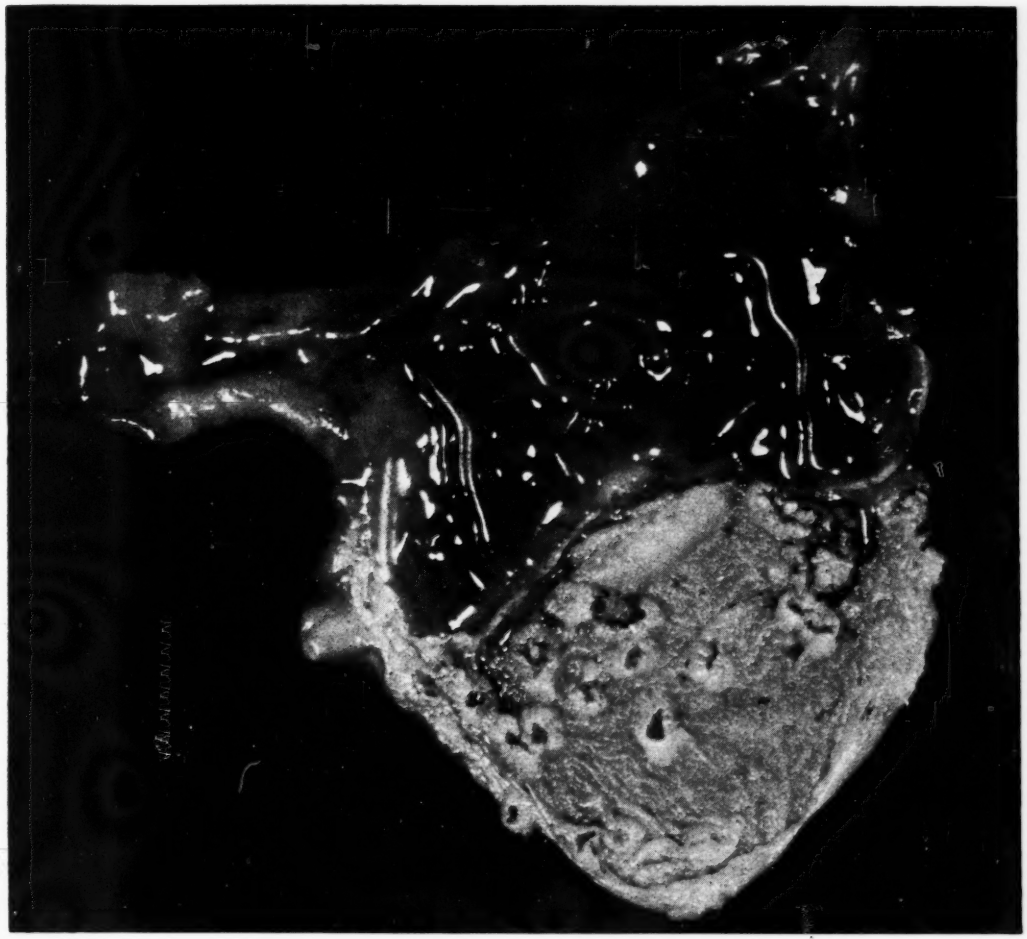

FIG. 1.—Stomach of 30 day old rat fed polished rice and kieselguhr for 86 days. Numerous lesions in the forestomach and hemorrhage in the glandular region of the stomach can be seen.

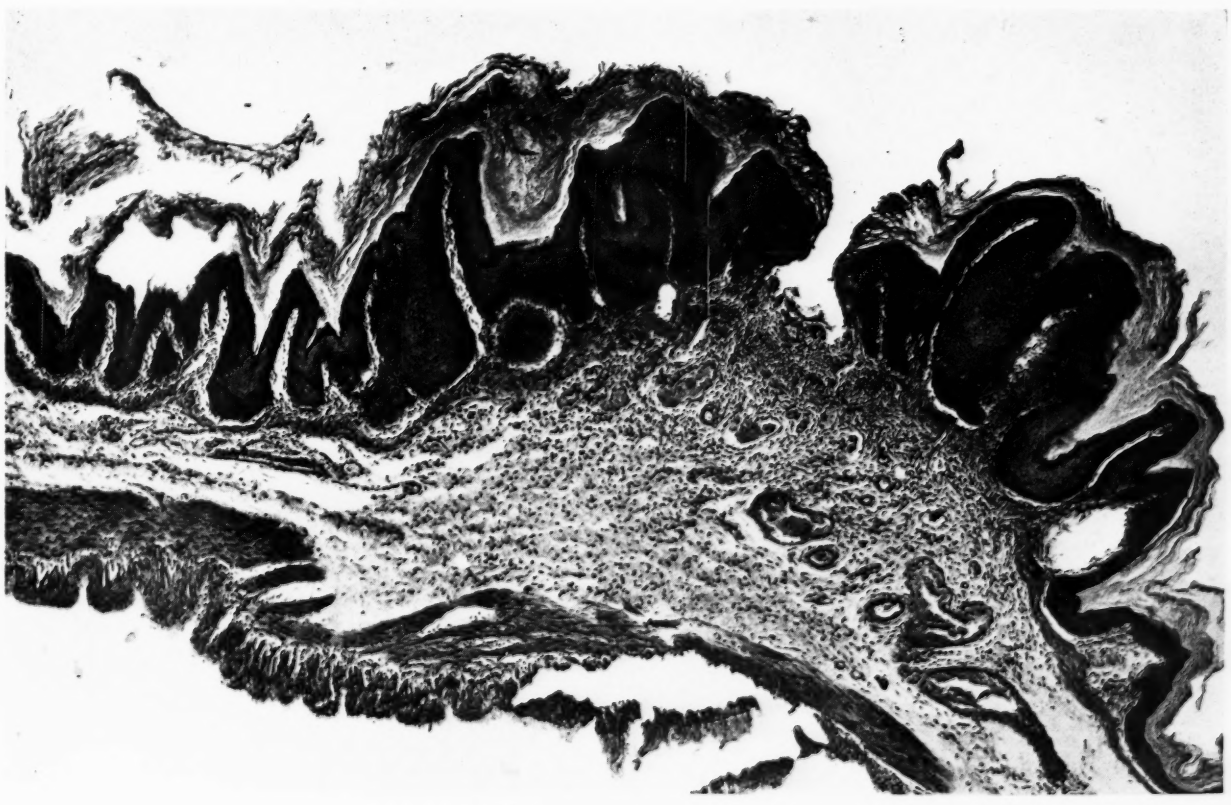

FIG. 2.—Photomicrograph of section through epithelial hyperplasia in rat's forestomach (Fig. 1) produced by polished rice and kieselguhr. Hematoxylin-eosin stain.

TABLE I: EXPERIMENTAL PRODUCTION OF GASTRIC LESIONS IN ANIMALS

| Year | Author | Animal | Carcinogenic agent | Diet | Lesion in forestomach | Authors' comments |
|---|---|---|---|---|---|---|
| 1913 | Fibiger (18) | Rat | *Gongylonema neoplasticum* | White bread | Papilloma, carcinoma | Infection |
| 1913 | Singer (53) | Rat, mouse | *Spiroptera* | White bread | Ulceropapilloma | Infection |
| 1914 | Fibiger (19) | Rat | *Gongylonema neoplasticum* | White bread | Papilloma, carcinoma | Infection |
| 1918 | Bullock and Rohdenburg (7) | Rat | Scarlet red, pine tar, or pig's bristles | Bread, turnips, carrots, and greens | Papilloma | Chemical or mechanical irritants with a deficient diet |
| 1919 | McCarrison (36) | Monkey | | Autoclaved rice, butter, and onion | Carcinoma | Vitamin B deficiency |
| 1920 | Fibiger (20) | Rat, mouse | *Gongylonema neoplasticum* | White bread | Papilloma, carcinoma | Infection |
| 1921 | Ishibashi and Ohtani (29) | Rabbit | Coal tar *per os* | Not mentioned | Adenoma | Chemical |
| 1923 | Buschke and Peiser (9) | Rat | Thallium chloride *per os* | Not mentioned | Papilloma | Chemical |
| 1923 | Buschke and Langer (8) | Rat | Coal tar *per os* | Not mentioned | Ulceropapilloma | Chemical |
| 1923 | Buschke and Langer (8) | Mouse | Methylcholanthrene *per os* | Not mentioned | Benign squamous cell papilloma | Chemical |
| 1924 | Pappenheimer and Larimore (41) | Rat | Wood shavings and hair | White flour and salt mixture | Keratosis, papilloma | Dietary deficiency and trauma |
| 1924 | Yokogawa (64) | Rat | *Gongylonema orientale* | Not mentioned | Epithelioma | Infection |
| 1926 | Fujimaki (23) | Rat | | Casein, dextrin, olive oil, salt mixture, and yeast | Hyperkeratosis, papilloma, carcinoma | Vitamin A deficiency |
| 1926 | Uyeyama (58) | Rat | | Rice and butter | Papilloma | Excessive fat and hunger |
| 1927 | Bonne (3) | Mouse | Tar painting | Not mentioned | Papilloma | Chemical |
| 1928 | Erdmann (16) | Rat | | Casein, dextrin, salt mixture, butter, yeast, and cod liver oil | Papilloma | Overrich in vitamins B and D |
| 1928 | Findlay (21) | Rat | | Casein, rice starch, salt mixture, and cod liver oil | Papilloma | Vitamin $B_2$ deficiency |
| 1928 | Fujimaki *et al.* (24) | Rat | | Casein, dextrin, salt mixture, butterfat, and yeast | Hyperkeratosis, papilloma, carcinoma | Excessive butterfat |
| 1928 | Büchner *et al.* (6) | Rat | Histamine injection | Not mentioned | Ulcer | Starvation and chemical |
| 1928 | Erdmann and Haagen (17) | Rat | | Casein, dextrin, salt mixture, olive oil, and yeast | Papilloma | Vitamin A deficiency |
| 1928 | Cramer (12) | Rat | | Adequate diet | Papilloma | Not caused by vitamin deficiency |
| 1929 | Voronoff and Alexandrescu (60) | Rat | Coal tar, wood fat, aniline oil, toluylenediamine *per os* | Not mentioned | Adenocarcinoma of the glandular stomach | Chemical |
| 1930 | Sugiura and Benedict (56) | Rat | | Casein, dextrin, salt mixture, olive oil, and yeast | Keratinization | Unbalanced diet |
| 1931 | Bisceglie (2) | Rat | | Casein, dextrin, salt mixture, olive oil, and yeast | Papilloma | Vitamin A deficiency |
| 1931 | McCarrison (37) | Rat | | Tapioca or rice with chilies, tamarind, and fish | Ulcer | |
| 1932 | Dalldorf and Kellogg (14) | Rat | | Casein, cornstarch, salt mixture, cod liver oil, and butterfat | Ulceropapilloma | Vitamin $B_1$ deficiency |
| 1932 | Twort and Bottomley (57) | Rat, mouse | Coal tar painting | Not mentioned | Papilloma | Chemical |
| 1932 | Hoelzel and Da Costa (25) | Rat | | White bread | Ulceropapilloma | Starvation and protein restriction |
| 1934 | Yoshida (65) | Rat | *o*-Aminoazotoluene *per os* | Polished rice and greens | Papilloma | Chemical |
| 1934 | Passey (42) | Rat | *Gongylonema neoplasticum* | White bread | Papilloma | Infection and vitamin A deficiency |
| 1935 | Passey *et al.* (43) | Rat | *Gongylonema neoplasticum* | White bread | Hyperkeratinization, papilloma | Infection and vitamin A deficiency |
| 1935 | Otsuka (40) | Mouse | Aminoazobenzene *per os* | Unpolished rice and greens | Papilloma | Chemical |
| 1936 | Sharpless (49) | Rat | | Casein, starch, salt mixture, butterfat, and yeast | Ulceropapilloma | Protein deficiency |
| 1936 | Howes and Vivier (27) | Rat | | White flour and salt mixture | Hyperkeratinization, papilloma | Dietary inadequacy |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1936 | Kinosita (30) | Rat | Tetramethylaminoazobenzophenone *per os* | Unpolished rice and greens | Papilloma | Chemical |
| 1936 | Waterman (61) | Mouse | Cholesterololeate *per os* | Not mentioned | Carcinoma, adenocarcinoma | Chemical |
| 1936 | Waterman (61) | Mouse | Benzpyrene in lard *per os* | Not mentioned | Squamous cell carcinoma | Chemical |
| 1936 | Reinhard and Candee (44) | Mouse | Dibenzanthracene in butter *per os* | Dog biscuit, cornmeal, butter, wheat, klim, $CaCO_3$ | Negative results | |
| 1936 | Ilfeld (28) | Dog | Dibenzanthracene in cholesterol pellets by injection | Not mentioned | Negative results | |
| 1936 | Ilfeld (28) | Ferret | Benzpyrene in cholesterol pellets by injection | Not mentioned | Negative results | |
| 1936 | Branch (4) | Mouse | Dibenzanthracene in benzene *per os* | Dog biscuit and vegetables | Negative results | |
| 1936 | Oberling *et al.* (39) | Mouse | 3,4-Benzpyrene in lard *per os* | Not mentioned | Benign hyperplasia | Chemical |
| 1937 | Hoelzel and Da Costa (26) | Rat, mouse | | White bread and flour | Ulceropapilloma | Starvation and protein restriction |
| 1937 | Cramer (13) | Rat | | Boiled maize, boiled rice, bread, cod liver oil, and marmite | Papilloma | Not to any specific dietary deficiency |
| 1937 | Cox (11) | Rat | | Casein, starch, salt mixture, lard, and yeast | Papilloma | Vitamin A deficiency |
| 1937 | Cook *et al.* (10) | Rat | Dibenzanthrazene in lard *per os* | Not mentioned | Negative results | |
| 1937 | Waterman (62) | Mouse | 1,2-Benzpyrene in lard *per os* | Not mentioned | Carcinoma | Chemical |
| 1937 | Sharpless (50) | Rat | | Casein, cornstarch, dextrin, salt mixture, butterfat, and yeast | Papilloma | Protein deficiency |
| 1938 | Roffo (45) | Rat | Heated animal fats | Bread, milk, and greens | Ulceropapilloma | Fats |
| 1938 | Roffo (45) | Rat | Irradiated cholesterol *per os* | Bread, milk, and greens | Carcinoma | Sterol |
| 1938 | Domagk (15) | Mouse | | Rice and olive oil, then regular diet, alternating | Papilloma, adenocarcinoma of the glandular stomach | Fats |
| 1938 | Matzner *et al.* (38) | Rat | Pepsin-HCl by injection | Yellow maize, milk powder, linseed oil meal, casein, salt mixture, and greens | Ulcer | Low protein |
| 1938 | Kinosita and Harada (31) | Rat | 4-Oxyazobenzene *per os* | Unpolished rice, dried fish, and greens | Papilloma | Chemical |
| 1939 | Van Prohaska *et al.* (59) | Mouse | Dibenzanthracene in lard *per os* | Not mentioned | Squamous epithelial papilloma | Chemical |
| 1940 | Sharpless (51) | Rat | | White flour, salt mixture, and butterfat | Ulceropapilloma | Protein deficiency |
| 1940 | Sharpless (52) | Rat | | White flour, salt mixture, butterfat, thiamin, riboflavin, pyridoxine, and nicotinic acid | Ulceropapilloma | Protein deficiency |
| 1940 | Fridericia *et al.* (22) | Rat | | Casein, rice starch, salt mixture, agar agar, lard, and yeast | Papilloma | Vitamin A deficiency |
| 1940 | Rusch *et al.* (48) | Rat, mouse | 3,4-Benzpyrene, 1,2,5,6-dibenzanthracene by injection | Not mentioned | Adenocarcinoma of the glandular stomach | Chemical |
| 1940 | Stewart (54) | Mouse | 20-Methylcholanthrene in petrolatum by injection | Purina dog chow | Papilloma, squamous cell carcinoma | Chemical |
| 1940 | Lorenz and Stewart (35) | Mouse | 20-Methylcholanthrene, 1,2,5,6-dibenzanthracene *per os* | Purina dog chow | Squamous cell carcinoma | Chemical |
| 1941 | Brunschwig and Rasmussen (5) | Rat | | Boiled rice alone, or casein, or heated lard | Ulceropapilloma | Unbalanced diet |
| 1941 | Stewart and Lorenz (55) | Mouse | 20-Methylcholanthrene by injection | Purina dog chow | Adenocarcinoma of the glandular stomach | Chemical |
| 1941 | Beck and Peacock (1) | Mouse | Heated fats *per os* | White bread, whole oats, milk, and green vegetables | Ulceropapilloma | Fats |
| 1941 | Roffo (46) | Rat | Tobacco tar *per os* | Bread, milk, and greens | Squamous cell carcinoma | Hydrocarbons |
| 1941 | Roffo (47) | Rat | Fuel oil distillate *per os* | Bread, milk, and greens | Papilloma, adenocarcinoma of the glandular stomach | Chemical |

TABLE II: SUMMARY OF INCIDENCE OF LESIONS OBSERVED IN THE FORESTOMACH OF RATS FED RICE ALONE OR RICE AND KIESELGUHR

| Experiment No. | Diet | Number of rats used | Age of rats, days | Duration of feeding, days | Number of animals with lesions | Number of animals without lesions |
|---|---|---|---|---|---|---|
| 1 | White rice and kieselguhr | 10 | 30 | 49–99 | 9 | 1 |
| 2 | White rice and kieselguhr | 11 | 75 | 98–116 | 11 | 0 |
| 3 | White rice | 27 | 30 | 54–148 | 13 | 14 |
| 4 | White rice | 24 | 75 | 65–156 | 16 | 8 |
| 5 | Brown rice | 12 | 30 | 54–75 | 0 | 12 |
| 6 | Brown rice and carrot | 30 | 75 | 88–200 | 0 | 30 |

Young rats, maintained on the diet of unpolished rice (brown rice) alone or unpolished rice plus fresh carrot, and surviving in fair health for periods of 75 to 200 days, showed no abnormalities in their stomachs. Certainly no new growths could be detected with the naked eye. Apparently the outer coating (bran) of the rice contained substances which inhibited the development of gastric lesions, since white rice without kieselguhr frequently produced tumors.

## SUMMARY

1. Rats fed polished rice developed areas of epithelial hyperplasia in the forestomach and diffuse hemorrhage in the glandular portion in about 60 days.

2. A deficient diet combined with kieselguhr produced more definite changes than could be elicited by polished rice alone.

3. Rats fed unpolished rice with or without carrot did not develop any abnormality in the stomach. No hyperplasia and no hemorrhage could be detected with the naked eye.

The author wishes to express his appreciation to Dr. C. P. Rhoads for his interest and valuable advice, and to Dr. Fred W. Stewart of the Department of Pathology for interpretation of the histological sections.

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# Studies on Rous Sarcoma Cells Cultivated in Vitro

## I. Cellular Composition of Pure Cultures of Rous Sarcoma Cells*

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(Received for publication March 23, 1942)

Although filterable fowl tumors, especially the Rous sarcoma, have been the object of extensive investigation during the last three decades, their cytohistological structure remains largely unknown. The method of tissue culture has done much towards clarifying the biological behavior of Rous sarcoma cells, but has barely added to our knowledge of their morphology.

We therefore found it desirable to undertake systematic studies on the structural characteristics of sarcoma cells and on the cellular composition of Rous tumors. Our investigations were carried out on pure strains of Rous sarcoma cells *in vitro*.

In the present communication we describe the cell types present in a pure culture of Rous sarcoma, and examine their developmental potencies and mutual relationships.

### MATERIAL AND TECHNIC

The experiments were carried out on pure strains of Rous sarcoma cells. They were derived from an 18 year old *in vitro* strain of Rous sarcoma, obtained through the courtesy of Dr. Albert Fischer of Copenhagen, and maintained in our laboratory for years with the regular addition of fragments of normal tissue.

The cultures were made according to the standard cover glass method of Carrel. We used chicken plasma and diluted embryonic extract as medium. The cell colonies were transferred every third day.

The cultures were examined microscopically both alive and after fixation. Cell colonies were fixed in Carnoy's fluid *in toto* and stained by Giemsa's method. Cultures for section were fixed in saline-formaldehyde and mixtures of Zenker's, Helly's Carnoy's, or Bouin's fluid, and stained with Giemsa's method, Heidenhain's hematoxylin, or hematoxylin-eosin. Fat staining was carried out with sudan III; vital staining with neutral red, in a dilution of 1:20,000. Phagocytic activity of the sarcoma cells was determined by adding carmine to the cultures.

* A preliminary note on this subject has been published (9).

### The Cellular Composition of Rous Sarcoma Cultures

Pure cultures of Rous sarcoma consist of two cell forms—spindle cells and round cells. Both spindle and round cells are always represented, but their relative proportion in different cultures is highly variable.

(*a*) *The spindle cells.*—The spindle cells (Fig. 1) in pure Rous sarcoma cell cultures resemble normal fibrocytes in all features. Their cytoplasm stains light bluish by Giemsa's method and is very transparent. The nucleus is regularly oval, pale, large, and possesses a fine membrane and one or two pale staining nucleoli; its chromatin is distributed in fine particles; its nucleoplasm is clear. The outgrowth pattern of fibrocyte-like cells is typical. They are either compact and radially arranged or formed into thin membrane-like layers. In the periphery of the cultures single cells are not infrequently detached and appear dispersed in the surrounding plasma.

(*b*) *The round cells.*—This cell type is represented by definitely basophilic cells of round or somewhat angular shape (Fig. 2). Their cytoplasm is homogeneous or finely granular and stains intensely with basic dyes. Generally it is free of vacuoles. The vesicular nucleus is relatively large, usually excentrically situated, round or oval, and provided with a thick, darkly staining membrane. It possesses one or two very prominent heavily staining nucleoli and a few chromatin particles which are finely dispersed, but may aggregate at times into small clumps. The round cells vary in diameter between 6 and 18 $\mu$. Mitotic figures are frequent; cells with two or more nuclei are common.

In cultures the round cells are evenly distributed between the peripheral and central portions of the growth area. They lie free or between proliferating spindle cells (Fig. 3). Not infrequently they appear to lie inside small hollows, easily seen in stained preparations (Fig. 4). Very often the basophilic round cells are found in a different plane from the spindle cells, appearing chiefly in the upper layers.

In living cultures the basophilic round cells appear as clear or as finely granular cell elements of a regu-

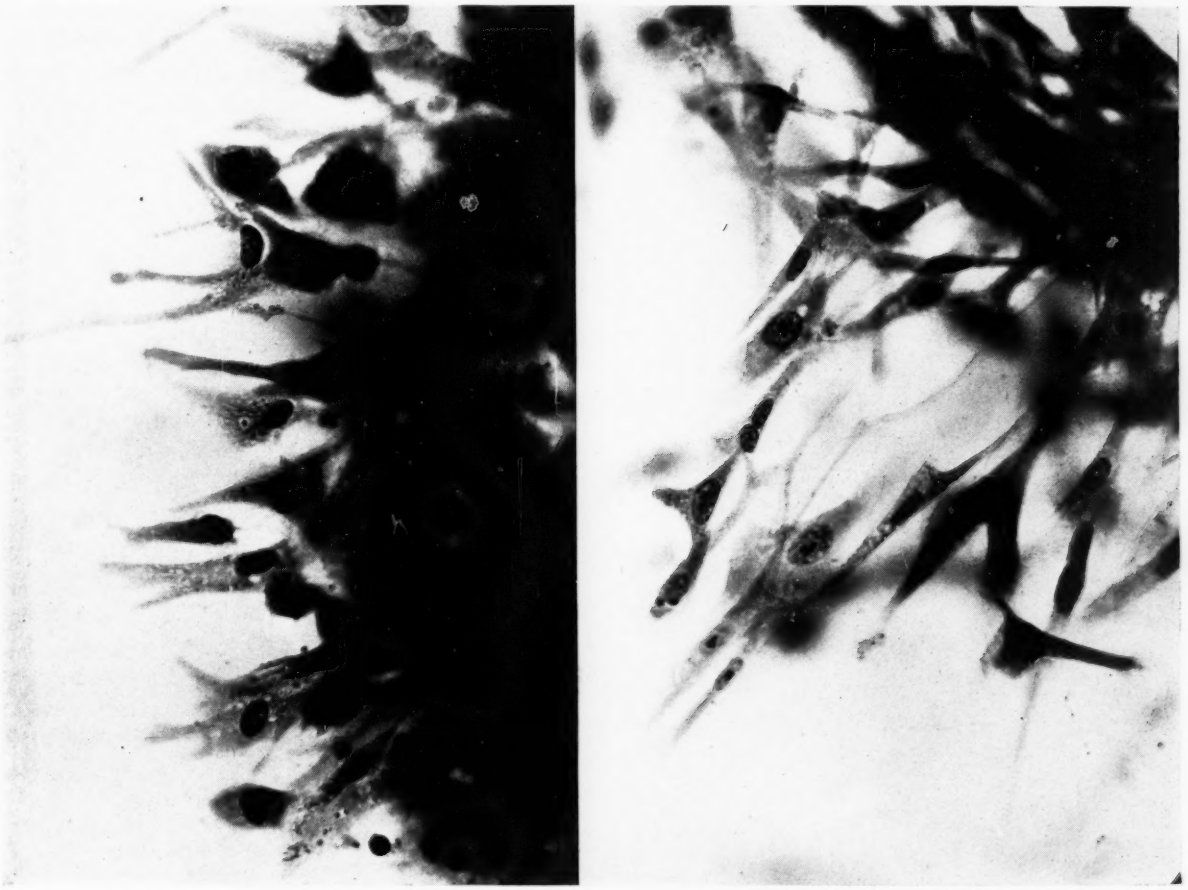

FIG. 1.—Fibrocyte-like cells in Rous sarcoma culture. Giemsa stain. Mag. × 400.

FIG. 2.—Basophilic round cells from Rous sarcoma culture. Giemsa stain. Mag. × 1200.

lar roundish shape. Their motility is very slight, and their movement resembles that of lymphocytes (Fig. 4). The cell outlines are sharp. The nucleus is barely visible. Carmine is not phagocytized by most of the round cells, but a few take up small amounts. The neutral red granules are of variable sizes, few in number, and irregularly distributed.

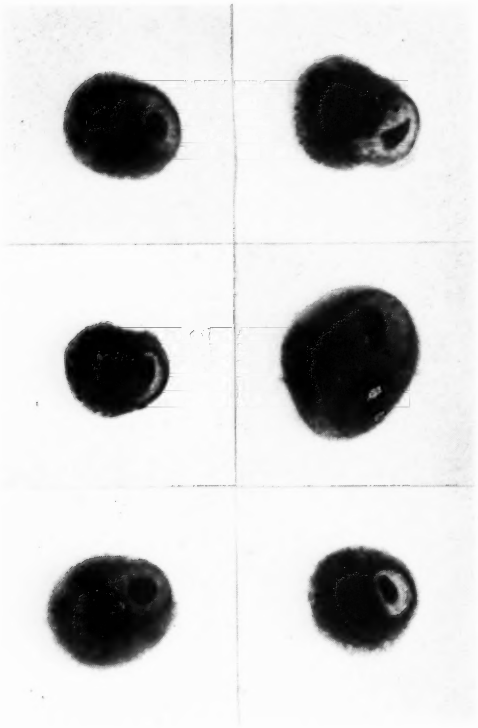

FIG. 3.—Basophilic round cells in Rous sarcoma culture. Giemsa stain. Mag. × 120.

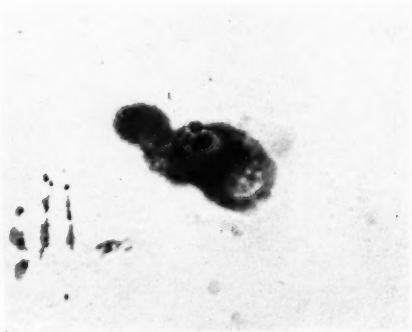

FIG. 4.—Basophilic round cell in movement. Giemsa stain. Mag. × 1200.

### THE MUTUAL RELATIONSHIP BETWEEN THE CELL ELEMENTS OF ROUS SARCOMA CULTURES

Cells which represent forms transitional between the fibrocyte-like cells and the basophilic round cells can be found in every culture. Their presence enables us to reconstruct, step by step, the process of transformation of the spindle cell into the basophilic round cell. In stained and unstained preparations of pure sarcoma cell cultures one can easily observe how cells of typical fibrocyte form contract here and there, singly or in groups, and take on the form of plump spindles. The cytoplasm, compared with that of the original spindle cells, appears denser and more basophilic. With further contraction the spindle cells ultimately turn into typical round cells of definitely basophilic character (Figs. 5, 6). During this transformation not only do the form of the cell and the structure of its cytoplasm change but the nucleus, too, is definitely altered. The pale, large fibrocyte nucleus is transformed into the round compact nucleus of basophilic round cells with a thick, darkly staining nuclear membrane and very prominent nucleoli.

The transformation of fibrocyte-like cells into basophilic round cells is intimately associated with the ability of sarcoma cells intensively to liquefy the plasma clot in which they grow. Liquefaction of plasma coagulum always begins around the individual sarcoma cells. The plasma layers on the cell border are loosened and digested so that cavities and duct-like formations, in which the cells nest, are produced. At first the liquefaction extends only to circumscribed areas but later it spreads through the entire plasma coagulum.

The transformation of the spindle cell into a basophilic round cell and the process of liquefaction concur both in time and space. The initial proliferation of the cell colony is predominantly that of spindle cells. It occurs in a medium as yet unaltered. After several hours the first circumscribed liquefactions of the medium, and simultaneously the first rounding off of the spindle cells, become demonstrable. Subsequently the cell transformation takes place, almost exclusively in the zone of liquefaction and in the cell layers adjoining it. Finally, when the whole of the plasma occupied by the cell colony is dissolved, all the spindle cells are found to have turned into round cells.

It is possible to initiate and accelerate the transformation of spindle cells into basophilic round cells by the addition of embryonic extract, which enhances the liquefying power of the cells and thus induces a rapid and complete conversion. If, on the other hand, embryonic extract is withheld, we obtain a culture consisting almost exclusively of spindle cells (Figs. 7, 8).

The reverse process—transformation of basophilic round cells into spindle cells—can also be easily and regularly observed. Basophilic round cells, floating within the area of liquefaction, stretch out parallel to the border of unliquefied plasma as soon as they become attached to the clot and turn into spindle cells. The newly formed spindle cells, plump and basophilic at first, may change into typical fibrocytes.

The transformation of round cells into fibrocytes is convincingly demonstrated by the following experiment: A Rous sarcoma culture whose growth zone consists exclusively of round cells is covered with fresh plasma. After 6 to 12 hours it may be seen that cells in this new and still unliquefied medium stretch out and turn into fibrocyte-like cells (Fig. 9).

pearance of a histiocyte nucleus. The ameboid cells originating from basophilic round cells accumulate carmine in large amounts and show numerous and coarse neutral red granules.

Ameboid cells deriving from basophilic round cells can also undergo a transformation into spindle cells. If the latter occurs in cell colonies cultivated in a medium

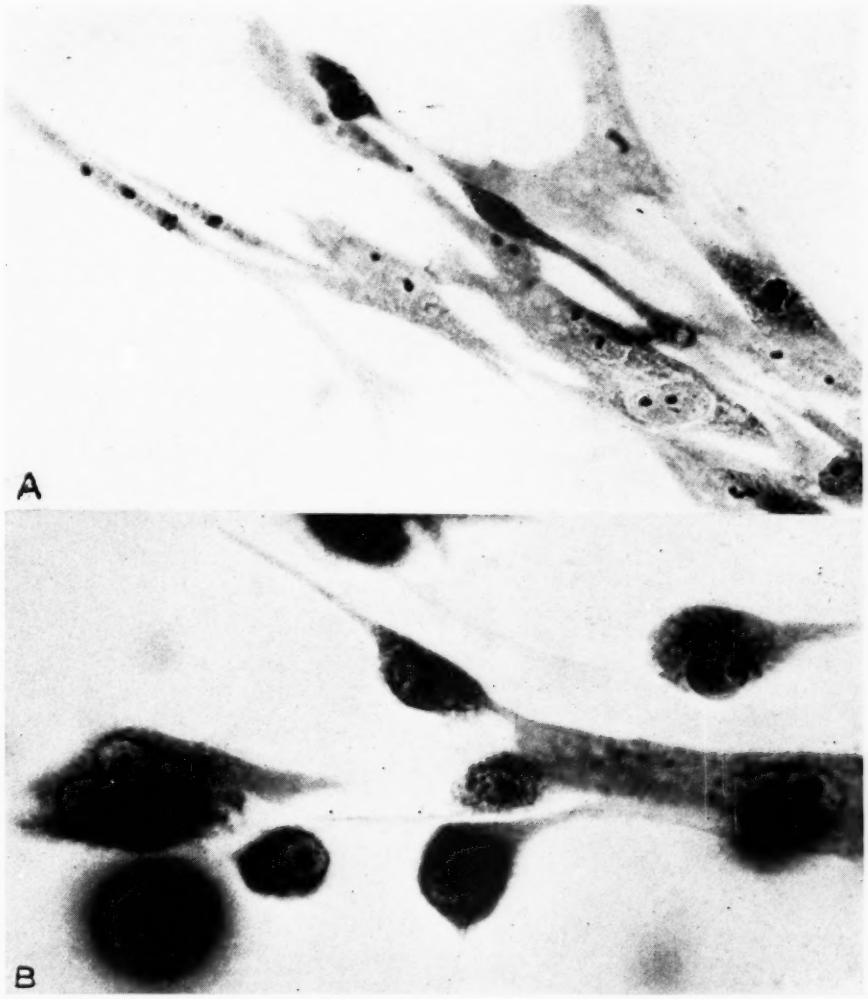


FIG. 5.—The transformation of spindle cells into basophilic round cells. Giemsa stain. (A) Mag. × 670, (B) Mag. × 990.

The basophilic round cells show a certain tendency in culture to evolve into cells with pronounced ameboid character. Not infrequently the very compact basophilic cytoplasm of the round cells may be observed to become progressively loose and vacuolated. This process is often accompanied by the appearance in the cytoplasm of intensely basophilic granular and filamentous structures. The cells lose their regular round shape and become polymorphous. Round or pointed filamentous or membrane-like active pseudopodia appear on the cell surface. In the course of these changes the cell volume is decidedly increased and the nucleus enlarges and assumes the typical ap-

containing carmine the ameboid cells which have stored the stain intensively become carmine-containing fibrocytes (Fig. 10).

Fischer (13) has stated that sarcomatous fibrocytes, unlike their normal prototypes, possess the ability of phagocytizing. This conclusion was based on the presence of carmine particles in fibrocytes of Rous sarcoma cultures grown in media containing carmine. Our observations, however, indicate that the carmine is phagocytized before the cells have been transformed into fibrocytes.

*The two chief cell forms occurring in pure Rous sarcoma cultures, spindle cells and basophilic round*

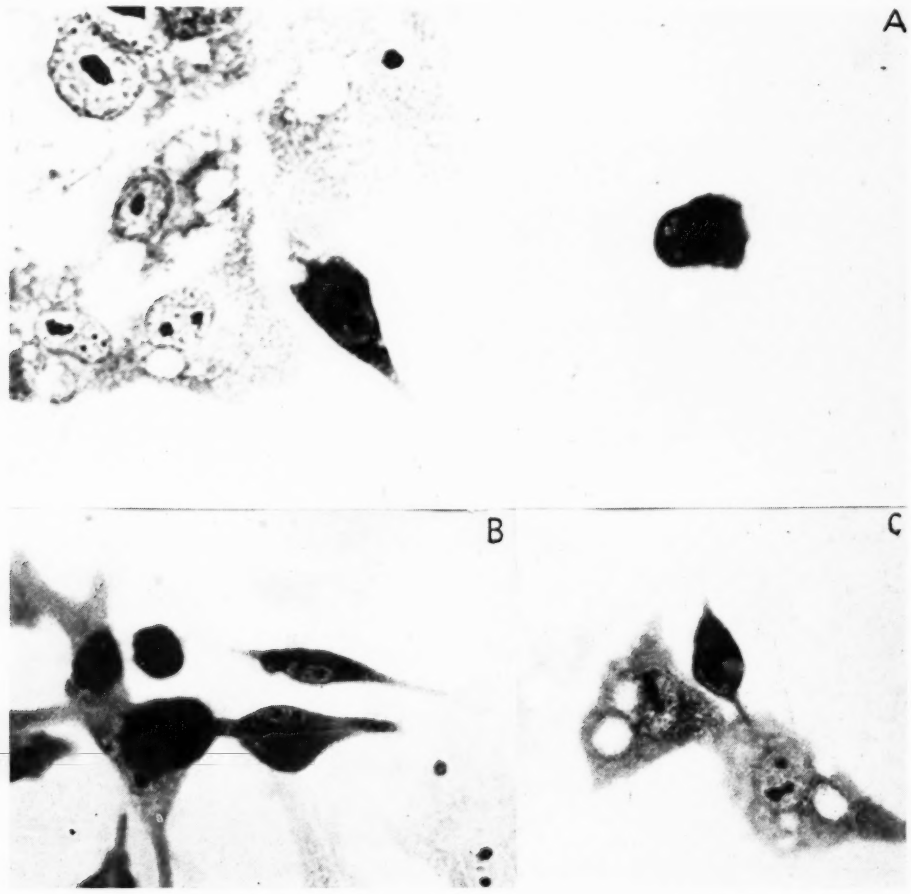


FIG. 6.—The transformation of spindle cells into basophilic round cells. Giemsa stain. (A) Mag. × 950, (B and C) Mag. × 675.

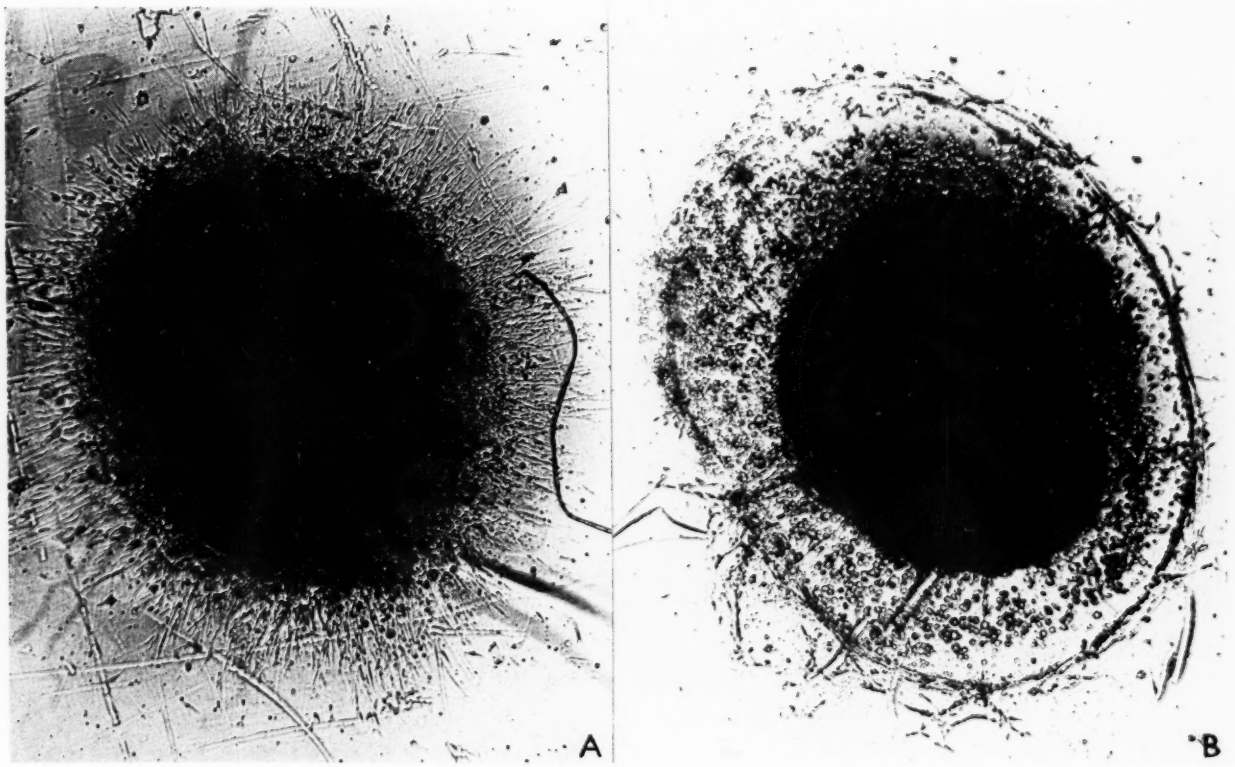


FIG. 7.—Experiment 12711/12712. Sister halves of a Rous sarcoma colony, 24 hours after cultivation: (A) without embryonic extract; (B) with addition of embryonic extract. Living. Mag. × 41.

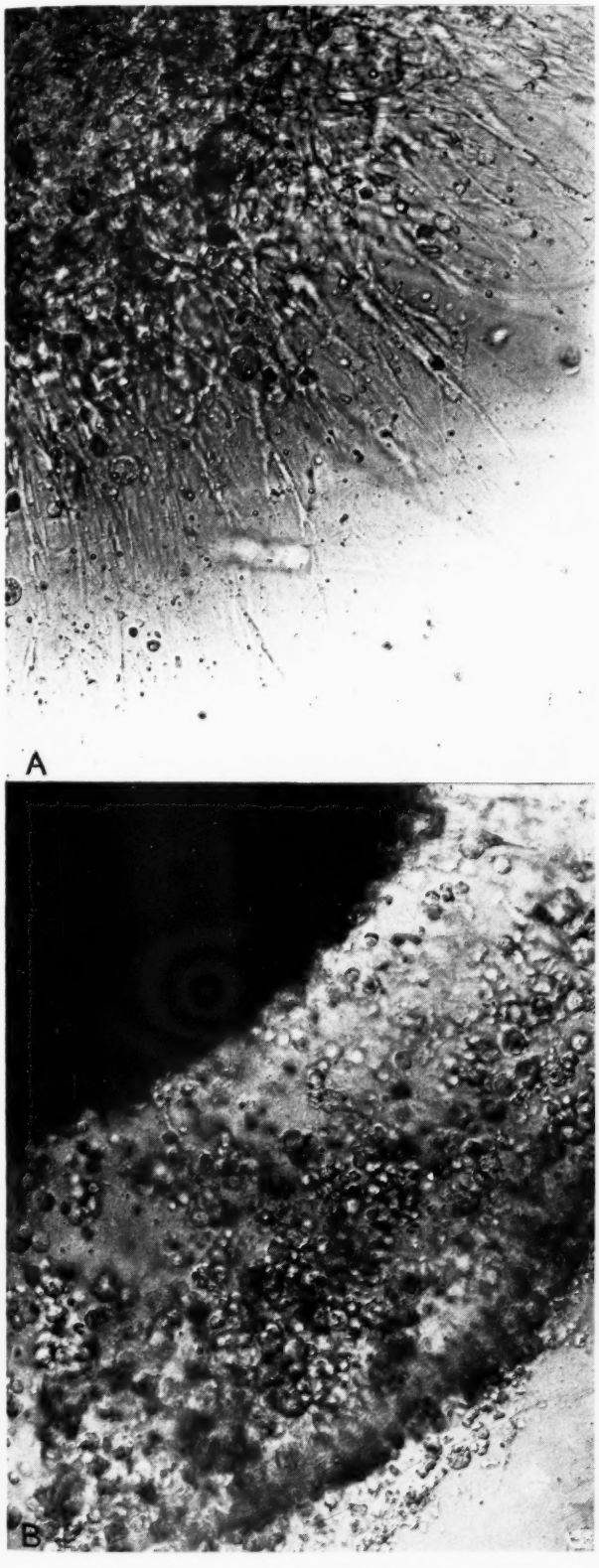


FIG. 8.—Experiment 12711/12712. Cultures shown in Fig. 7. Mag. × 140.

*cells, are variants of one cell type. Transitional forms, met in culture, indicate that the basophilic round cells arise from fibrocyte-like cells. This process of transformation is reversible; the basophilic round cells can, in turn, change back into spindle cells. The basophilic round cells may also be the source of large ameboid cell forms. From all appearances it seems that the process of transformation depends upon the physical state of the plasmatic medium.*

## DISCUSSION

It was first pointed out by Carrel (3, 4) that two cell types are characteristic of sarcoma cultures: fibrocyte-like cells and free round cells. The latter he designated as monocytes or macrophages. Carrel stated that the fibrocytes and macrophages of sarcoma cultures are not different from the corresponding normal cell forms. Occasionally, however, sarcomatous macrophages were found in a state of advanced degeneration. "The cytoplasm of some monocytes became full of vacuoles and granulations, their shape more or less rounded, and the rate of migration and multiplication decreased" (5). Sarcomatous macrophages were regarded, therefore, as a particularly labile, short lived cell type.

Fischer (10) adopted Carrel's classification and differentiated the "macrophages" into small, medium, and large ameboid forms (11). The smallest free cells are very motile and granular; their cytoplasm stains deeply. The medium sized cells have clearer cytoplasm and are also distinctly motile. The largest cells are of slow movement; their cytoplasm is highly granular and often contains vacuoles in characteristic arrangements. Mitotic cell division does not occur. In a later paper (13), however, Fischer stated that the sarcomatous macrophages are similar to normal monocytes or macrophages from cultures of blood cells or spleen.

A similar classification of the cells in Rous sarcoma cultures into spindle cells and macrophages was accepted by Borrel (1) and Zweibaum (27). Borrel found that both kinds of cells contained eosinophilic granules or masses which he believed to be characteristic of the Rous sarcoma. These cells were often notably hypertrophic and in this case contained numerous large mitochondria. According to Zweibaum, the fine structure of the sarcomatous macrophage differs from that of the normal in the following points: (a) constant absence of any structure in the living nucleus and the absence of nucleoplasmosomes; (b) characteristic and constant differentiation of the centrosphere; (c) considerable increase in chondriom, modified in its constitution; (d) increase in lipoids; and (e) noteworthy imbibition of the fundamental cytoplasm.

Neither Borrel nor Zweibaum presented any particulars concerning the mutual relationship between the cell types occurring in sarcoma cultures. Carrel (6) and Fischer (12) observed that the ameboid cells ("macrophages") of Rous sarcoma cultures can occasionally turn into fibrocytes. This transformation is correlated with a loss of malignancy.

Our studies have established that in addition to fibrocyte-like cells only one other cell type is an essential and obligatory constituent of Rous sarcoma cultures. This is the basophilic round cell. It is always present in pure cultures of Rous sarcoma cells, but at different periods in the development of the culture the degree of its participation in the composition of the culture varies. In the first hours of development the basophilic round cell is sparsely represented; after 2 to 3 days it predominates. The constant presence of the basophilic round cells together with the spindle cells is a distinct characteristic of Rous sarcoma cultures.

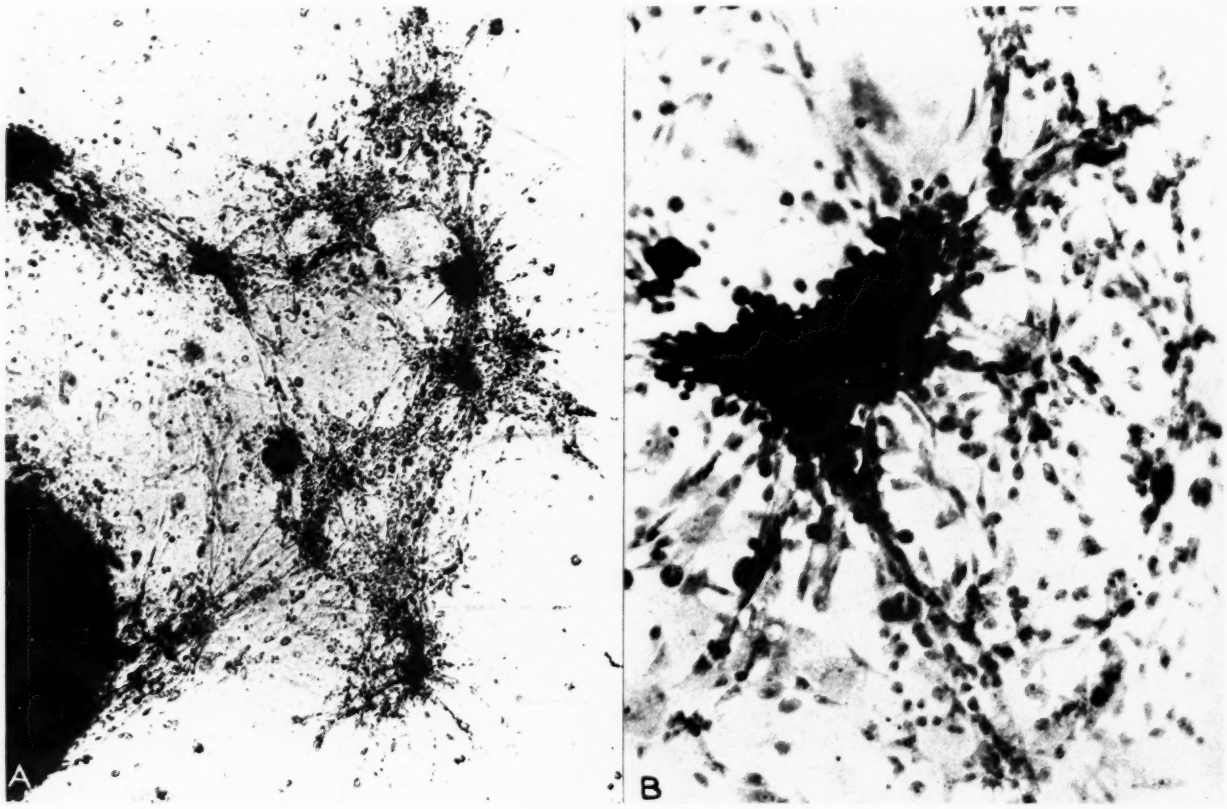


FIG. 9.—Experiment 12698/15111. A Rous sarcoma culture composed exclusively of basophilic round cells is overlayered with fresh plasma. After 18 hours the round cells are found to be stretched. Living. (A) Mag. × 31, (B) Mag. × 367.

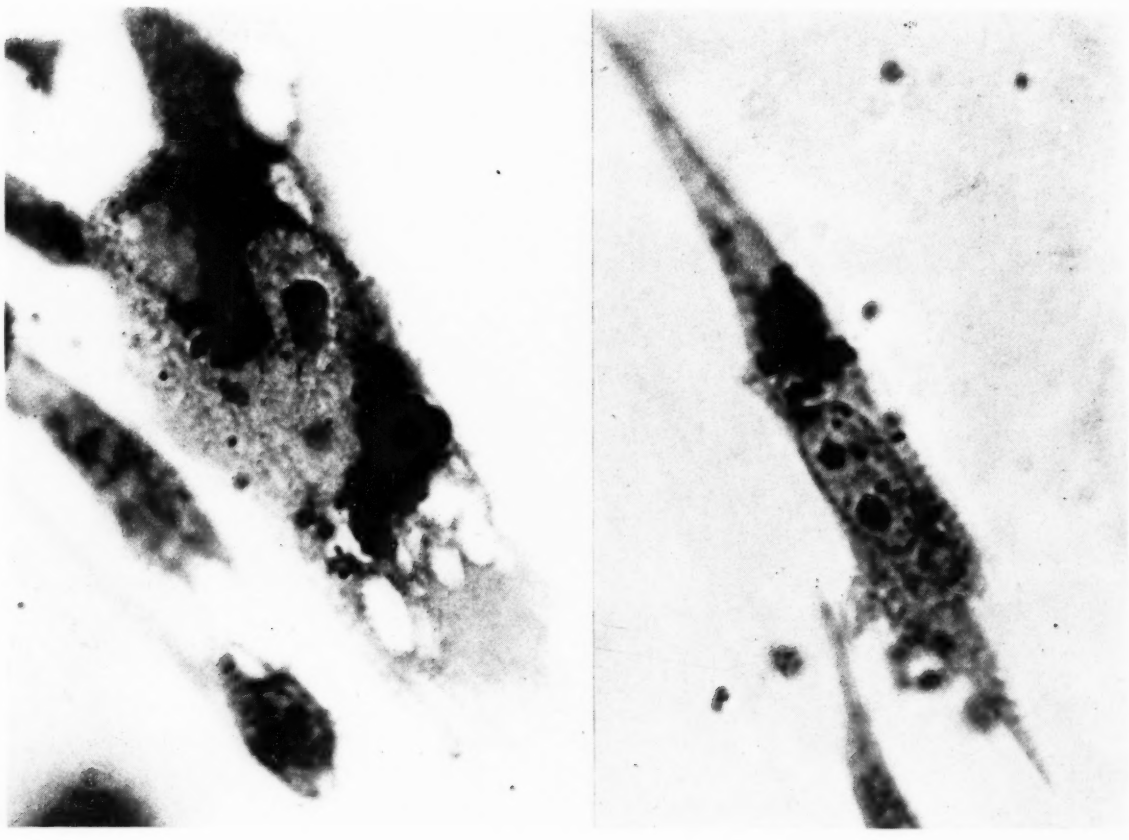

FIG. 10.—Experiment 12838. Round cells cultivated in media containing carmine phagocytize the latter. In a new medium they stretch and turn into carmine-containing spindle cells. Giemsa stain. Mag. × 1900.

Teutschlaender (25, 26) called attention to the fact that lymphocyte-like cells can be found in filterable chicken sarcoma. In sections of chicken sarcoma he found lymphocyte-like cells with round chromatin-rich nuclei; he called these cells *indifferente Blastemzellen* and identified them with the macrophages that Carrel and Fischer found in sarcoma cultures.

From Fischer's description of the ameboid cells in Rous sarcoma cultures (11) one can assume that among the various cell forms which he observed and which he designated as belonging to the macrophage type, he probably saw also the basophilic round cell. He said, for instance, that the ameboid cell of the small type stained deeply with azure II and methylene blue; whether these cells also possessed other characteristics of the basophilic round cell was not indicated.

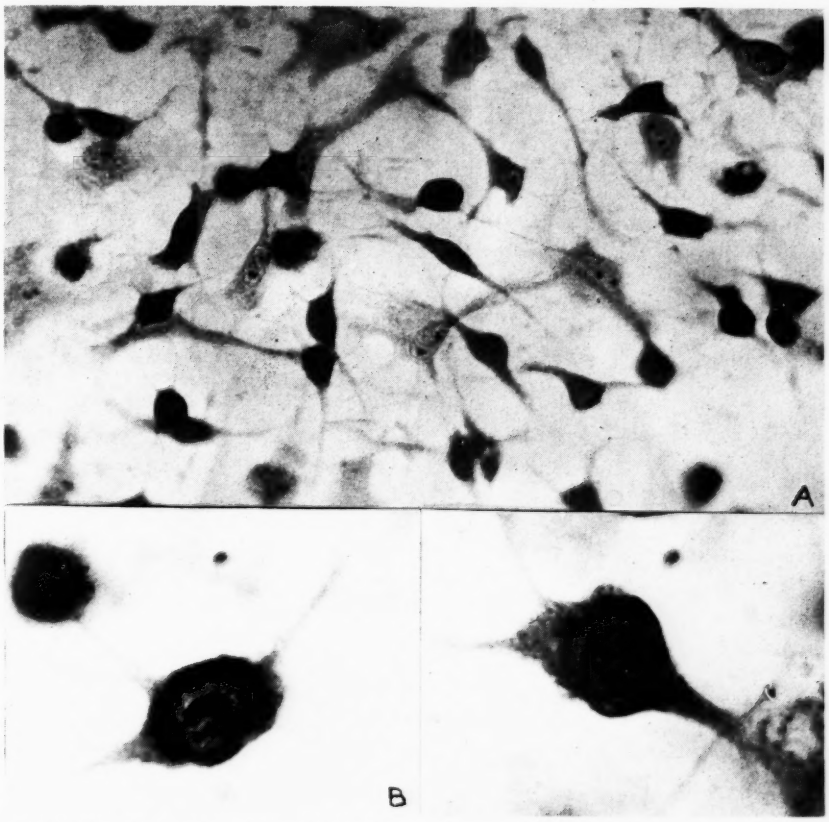


FIG. 11.—Contracted spindle cells in normal fibrocyte culture. Giemsa stain. (A) Mag. × 510, (B and C) Mag. × 1000.

Ludford no doubt saw the basophilic round cell and indeed designated it, in contrast to normal macrophages, as the malignant element of the sarcoma culture. In his work (18) on the differential reaction to trypan blue of normal and malignant cells *in vitro,* which contains studies on various tumors, he said, in the section devoted to Rous sarcoma culture, that in addition to macrophages which wander out from the explants he found another cell form that did not emigrate and which could therefore be studied in serial sections only. Ludford said nothing of the structural characteristics of these cells, but mentioned that the deeply stained nuclei contained relatively large nucleoli. His illustrations (Plate 48, Fig. 54) were, however, very suggestive.

The basophilic round cell represents a sharply delimited cell type whose main characteristics are a definitely basophilic and usually homogeneous cytoplasm free of, or poor in, pseudopodia, and a roundish, relatively large vesicular nucleus with a well defined membrane and heavy nucleoli. The basophilic round cell is slightly mobile and phagocytizes little if at all. Though it can itself turn into an ameboid macrophage the basophilic round cell is decidedly different in the character of its cytoplasm, nuclear strucure, and general behavior from the motile and actively phagocytizing macrophages occurring in tissue cultures of blood, spleen, and so forth. It shows, rather, certain similarities to the lymphocyte in nuclear and cytoplasmic structure and even more to the hemocytoblast of cultures of bone marrow and leukotic blood cells.

As we have stated in detail above, the basophilic round cell arises through contraction of the spindle cell. This process appears to be causally associated with the pronounced ability of the sarcoma cell to digest the plasma coagulum. The change induced by the sarcoma cell in the physical state of the medium inevitably provokes a change in the cell's form. Stretched out in the semisolid medium, the cell becomes round when the plasma is liquefied.

It seems important in this connection to note that normal fibrocytes, too, can undergo transformation into basophilic round cells. In cultures of normal chicken fibrocytes the plasma coagulum may occasionally be observed to loosen, owing to gradually progressing but limited liquefaction. Fibrocytes in these areas form a loose cell network consisting of contracted, dark staining, angular, stellate cells which are joined by long protoplasmic processes (Fig. 11). With further lique-

faction the protoplasmic connections are broken and the fibroblast is transformed into a free round cell which, with its decidedly basophilic protoplasm, round dense nucleus, and heavy nucleoli, *is difficult to distinguish from the basophilic round cell of the sarcoma culture* (Fig. 12).

Rous and Jones (24) were the first to show in a convincing manner that the transformation of fibrocytes into round cells is causally associated with a change in the physical state of the milieu. These authors submitted cultures of connective tissue cells, growing in plasmatic medium, to the action of trypsin. In these conditions the living spindle cells assumed a spherical

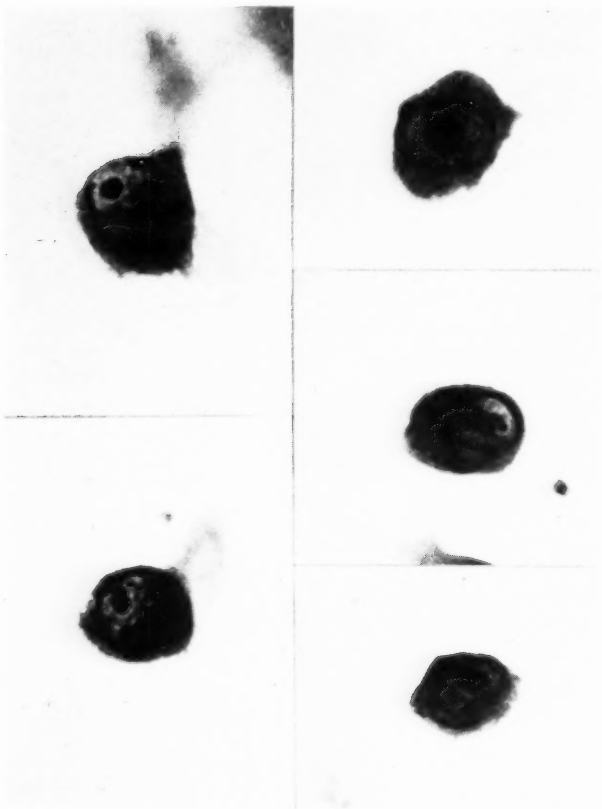

FIG. 12.—Basophilic round cells arising from contracted spindle cells in normal fibrocyte culture. Giemsa stain. Mag. × 153.

form and ultimately transformed into perfectly round cells with basophilic cytoplasm which had "a resemblance to the mononuclear series of the blood." The formation of round cells from fibrocytes and reticulum cells was later described by Hegue (15), Busse (2), Maximow (20–22), Chlopin (8), de Haan (14), and by von Möllendorff (23).

The similarity between the basophilic round cells arising from fibrocytes in normal cultures and from spindle cells in sarcoma cultures does not appear to be accidental. The mechanism of the transformation of the spindle cell into a basophilic round cell is apparently the same in both these cases. In both, the round cell is formed as a result of the contraction of the spindle cell, and, too, the process of cell contraction is preceded by a change in the physical state of the milieu. The difference between them is only in the constancy and intensity of the transformation process. In normal cultures liquefaction of the coagulum is rare, and when it occurs is slight. In sarcoma cultures, on the other hand, liquefaction is regular, and always intense. Consequently *the process of transformation is exceptional in normal fibrocyte cultures, but the rule in sarcoma cultures.* The multiplicity of the forms in which the sarcoma cell appears *in vitro* is no doubt associated with the essential ability of the sarcoma cell to liquefy the plasma clot in which it lives. In this manner the sarcoma cell itself creates the conditions which determine its polymorphism.

In connection with the question of the cellular composition of Rous sarcoma cultures, discussion has arisen as to which of the various constituents is the actual carrier of malignancy. Carrel (3, 4, 7) and Fischer (10) believe that the ameboid cells ("macrophages" in the terminology of these authors) are the malignant elements of the sarcoma cell colony, and that the spindle cells lack neoplastic properties. Lewis (17) opposes this view and suggests the possibility that the spindle cell, and not the macrophage, is the malignant element of the sarcoma culture. Ludford (19), too, has been later led by his studies on the maintenance of Rous agent *in vitro* to the opinion that the fibrocytes, and not the macrophages, are the actual malignant elements, since only the former have been found by him to be susceptible to the Rous agent.

An analysis of the cellular composition of the Rous sarcoma culture and of the genetic relationship of its various cell types yields definite evidence that the Rous sarcoma cell culture consists not of different cell types, but of only one, which, depending upon external conditions, can assume various aspects. This mesenchyme cell, which possesses the ability to appear now in the form of a fibrocyte-like cell and now in that of a basophilic round cell, is the actual carrier of the Rous agent.

## SUMMARY

A pure culture of Rous sarcoma consists of two cell forms—spindle cells and basophilic round cells. Both are variants of one cell type. The basophilic round cell arises from a fibrocyte-like cell and may also turn into the latter. It may also become a source of large ameboid cell forms. The polymorphism of the sarcoma cell is due to the changes which it induces in the physical state of the medium. Both cell forms—the spindle and the basophilic round cell—are considered to be carriers of the Rous agent.

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# Further Experiments on the Extraction of a Carcinogenic Factor from Human Cancerous Tissues

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(Received for publication June 10, 1942)

In a preliminary report (6), an announcement was made that a lipoid extract of two primary human mammary cancers had produced sarcomas at the site of subcutaneous injections in mice of a well known, low spontaneous tumor strain. Since that time additional experiments have been performed, and although the further results are still inconclusive it seems desirable to make a progress report at this time.

This investigation was started on the assumption that human cancerous tissue might contain a carcinogenic factor resembling the known synthetic compounds. The possibility did not seem unlikely when one noted the close relationship between these agents and the normal steroid compounds of the animal body. The property of fat-solubility, common among the known carcinogenic agents, stood out as a characteristic that might also be common to a hypothetical human carcinogenic factor. This suggested the extraction of human cancerous tissue with fat solvents as a method that might yield a crude extract in which carcinogenic activity could be demonstrated by subcutaneous injection into mice of known low spontaneous tumor incidence.

Several workers have reported experiments designed to demonstrate the existence of a fat-soluble carcinogenic factor in the tissues of cancer patients. Schabad (4, 5, 7) in 1937 reported an active extract made from the livers of patients who died of cancer primary in other organs. Steiner (8, 9) described similar results. des Ligneris (1) and Hieger (2) reported tumors following the injection of extracts of primary liver cancer and of noncancerous livers from South African Bantu natives, and Hieger (3) the production of a sarcoma at the site of injection of an extract of mouse mammary carcinoma.

The author has limited his work to primary cancerous tissue, mainly of the mammary gland, but this report will include some experiments on rectal cancer and on osteogenic sarcoma.

## MATERIALS AND METHODS

### The Varieties of Human Tissue Tested

*Primary mammary cancer.*—Nine specimens, obtained at operation. The primary tumor or the entire breast after having been freed of skin and pectoral muscle was quickly sliced into small pieces and covered with cold acetone.

In the experiments reported herein, three types of surgical specimens have been used for extraction. The results will suggest that the following classification of specimens for extraction is important.

Type A: The primary tumor. In this case the surrounding mammary tissue and fat were dissected free and discarded.

Type B: Specimens in which the entire primary tumor had been removed for premastectomy biopsy, leaving only the uninvolved or secondarily involved mammary tissue and fat for extraction.

Type C: Specimens in which the primary tumor was present. The entire specimen, neoplasm and surrounding tissues, was used for extraction.

*Noncancerous mammary tissue.*—Four specimens, obtained either from autopsies or from operations. All were carefully examined in the gross for possible early cancer and all suspiciously firm areas were examined microscopically. No cancerous tissue was found. The tissues were prepared and extracted in the same manner as were the cancerous specimens. These four specimens were obtained as follows:

H.M. Nonca. #1. Obtained at autopsy. A 40 year old white woman who died of hypertensive cardiovascular renal disease.

H.M. Nonca. #2. Obtained at autopsy. A 47 year old white woman who died of diabetes mellitus, and was found to have a chromophobic adenoma of the pituitary gland.

H.M. Nonca. #3. Obtained at autopsy. A 25 year old white woman who died of pulmonary tuberculosis complicated by miliary spread of the disease.

H.M. Nonca. #4. Obtained at operation. A 49 year old white woman who is now living and well. Simple mastectomy was performed because of painful breasts due to chronic cystic disease. Diagnosis confirmed by microscopic examination.

*Primary rectal cancer.*—Two specimens, obtained at operation. The bowel contents were thoroughly washed out, and the cancerous area and neighboring uninvolved bowel sliced into small pieces and covered

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with cold acetone. These specimens were comparable to the mammary cancerous tissues of type C.

*Primary osteogenic sarcoma of the femur.*—One specimen, also obtained at operation. The primary area was dissected free from the uninvolved parts. The noncalcified tumor was sliced, the calcified bony portions were fragmented by crushing in a vise, and the whole was covered immediately with cold acetone.

### Method of Extraction

The material covered with acetone was extracted in a Soxhlet apparatus until the solvent returned colorless or until a sufficient time of exposure to the solvent had elapsed (10 to 24 hours). The following solvents, in order, were used: acetone, ethyl ether, petroleum ether (b.p. 35–60° C.), absolute ethyl alcohol. After extraction with the solvents, the separate extracts were combined, and the solvents were distilled off at reduced pressure. The combined residue of yellow, oily material, called the whole extract, was used as such for injection. Further sterilization of the extract was deemed unnecessary because of the antiseptic properties of the solvents.

The quantity of whole extract obtained from a given amount of tissue was variable, depending upon the relative amounts of fat and functional tissue. In a fairly well circumscribed mammary carcinoma in which it was possible to remove most of the surface fat, 21 gm. of cancerous tissue yielded 8.8 gm. of whole extract.

### Preparation of Fractions of the Whole Extracts

*Nonsaponifiable fraction.*—The whole extract was saponified in a 10 per cent alcoholic potassium hydroxide solution. The mixture was refluxed on a water bath for 6 hours. The nonsaponifiable fraction was then removed in one of two ways. (a) After the solution had been diluted with water or 12 per cent saline to an alcoholic concentration of 20 per cent or less, it was extracted with ether and with petroleum ether and evaporated. (b) The saponified solution was concentrated to a thick syrup by distillation at reduced pressure. This was mixed with anhydrous sodium sulfate and further dehydrated to a dry powder in an Abderhalden dryer. When thoroughly dry, the material was extracted with petroleum ether (b.p. 20–40° C.) and evaporated.

*Digitonide and nondigitonide fractions.*—These were made by repeated precipitation of the nonsaponifiable fraction with an alcoholic solution of digitonin, which resulted in a digitonide and a nondigitonide fraction. The digitonide was freed of digitonin by dissociation in cold pyridine and extraction of the digitonide fraction with ether.

*Noncarbonyl fraction.*—This was made by removal of the carbonyl compounds from the nonsaponifiable fraction through the use of Girard's reagent, to remove the ketonic estrogenic compounds.

The nonsaponifiable, digitonide, nondigitonide, and noncarbonyl fractions were moistened with sesame oil and the suspension was injected into mice. Sesame oil was not used for the whole extracts.

### Animals

The mice used in this experiment were of the C57 black strain. The strain has been kept pure by brother-to-sister matings only. All mice were 2 months of age or younger at the time of the first injection of the extract. Unless otherwise stated, males and females were kept separate in one gallon size glass cookie jars, 4 to 6 mice to each. The diet consisted entirely of Purina dog chow pellets and was fed in wire mesh food holders. Water was available at all times. The bedding was autoclaved wood shavings.

### Method and Site of Injection

The extracts were tested by subcutaneous injection only. In females, and in some males, the injections were made in such a way as to infiltrate the subcutaneous area beneath the left and right caudad nipples; in the males, the location of the nipple was approximated. Most of the male animals, however, were injected subcutaneously in the right and left anterior axillary regions. The mice received 2 to 4 injections alternately on the left and right sides at intervals of 1 to 3 weeks. The amount of extract for each injection varied between 0.2 cc. and 0.4 cc.

## RESULTS

### Mammary Cancerous Tissues

Details of the results of these experiments are given in Table I.

Tumors occurred at the site of injection only in animals treated with the whole extracts made from primary tumors, type A. Of 36 animals treated with such extracts, 7 developed sarcomas at the site of injection. Figs. 1 and 2 are photographs of 2 tumor-bearing mice, selected to show tumors in the two sexes and at the different sites of injection. Figs. 3 and 4 reproduce the histological appearance of the tumors. Each of the 7 has been found readily transplantable to mice of the same strain, and has been carried through 4 to 6 generations.

In the series receiving extracts of mammary cancerous tissues of types B and C, no tumors occurred at the site of injection. A number of animals in these series developed an anal lesion followed by a prolapse of the

rectum. This was first noted about the 5th month after injection, when animals were found to be passing blood *per rectum*. Except for the bleeding, the animals appeared to be in good health. There were no signs of other intestinal disorder nor did the appetite or general activity of the animals seem unusual. Two to 3 weeks following the onset of bleeding there appeared a pale red enlargement of the ventral margin of the anus. This slowly developed until, after about 3 more weeks, the enlargement had spread completely around the anal ring, being largest on the ventral half. Finally the anal orifice was so obstructed that a complete prolapse of the rectum occurred and the animals then lost weight rapidly and soon died. The earliest appearance of these anal lesions was 168 days after the first injection, the longest interval was 567 days, and the average time 394 days.

Table I: Results with Cancerous Mammary Tissue

| | Animals | | Injections | | Number developing tumors at site of injection | Number developing anal lesions | Animals still living | Duration of experiment, months |
|---|---|---|---|---|---|---|---|---|
| | Number | Sex | Number | Amount, cc. | | | | |
| WHOLE EXTRACT | | | | | | | | |
| Type A* | | | | | | | | |
| H.M.Ca. #1 | 8 | 4♂ | 4 | 0.2 | 2—425, 440 days† | 0 | 0 | 24 |
| | | 4♀ | 4 | 0.2 | 1—282 days | 0 | 0 | 24 |
| H.M.Ca. #2 | 5 | 5♀ | 2 | 0.2 | 2—229, 319 days | 0 | 0 | 24 |
| H.M.Ca. #14 | 23 | 23♂ | 4 | 0.4 | 2—328, 370 days | 0 | 14 | 14 |
| Type B | | | | | | | | |
| H.M.Ca. #3 | 33 | 23♀ | 1 | 0.3 | 0 | 8 | 0 | 20 |
| | | 10♀ | 2 | 0.3 | 0 | 4 | 0 | 20 |
| Type C | | | | | | | | |
| H.M.Ca. #4 | 6 | 6♀ | 2 | 0.2 | 0 | 4 | 0 | 19 |
| H.M.Ca. #7 | 12 | 12♂ | 3 | 0.2 | 0 | 0 | 9 | 16 |
| NONSAPONIFIABLE FRACTION | | | | | | | | |
| Type B | | | | | | | | |
| H.M.Ca. #3 | 18 | 18♀ | 2 | 0.2 | 0 | 2 | 0 | 17 |
| Type C | | | | | | | | |
| H.M.Ca. #4 | 10 | 10♀ | 3 | 0.2 | 0 | 7 | 0 | 17 |
| H.M.Ca. #13 | 12 | 12♀ | 2 | 0.2 | 0 | 1 | 10 | 15 |
| DIGITONIDE FRACTION | | | | | | | | |
| Type B | | | | | | | | |
| H.M.Ca. #6 | 6 | 6♂ | 3 | 0.2 | 0 | 0 | 3 | 17 |
| NONDIGITONIDE FRACTION | | | | | | | | |
| Type B | | | | | | | | |
| H.M.Ca. #6 | 6 | 6♀ | 3 | 0.2 | 0 | 4 | 0 | 17 |
| NONCARBONYL FRACTION | | | | | | | | |
| Type C | | | | | | | | |
| H.M.Ca. #8 | 18 | 18♀ | 2 | 0.2 | 0 | 1 | 16 | 15 |

* Type A: Primary tumor only.
Type B: Whole breast, primary tumor absent.
Type C: Whole breast, primary tumor present.
† Number of days from first injection to appearance of tumor.

The gross appearance of early and late stages of these anal lesions is shown in Figs. 5 and 6. The microscopic appearance is shown in Figs. 7 and 8. There is an increase in the number and size of the glandular structures resembling the sebaceous glands

normally found between the internal and external sphincter muscles. The cells of the hyperplastic tissues are uniform and evenly stained, without evidence of malignant growth. Proliferation appears to be in progress only at the outer edge of each follicle, where an occasional mitotic figure is seen; the successive rows toward the center show greater degrees of differentiation, resembling mature secretory cells. The prolapsed bowel is infiltrated with polymorphonuclear leucocytes and is ulcerated in some areas.

FIG. 1.—Tumor at site of injection of extract of primary human mammary carcinoma. Male. Mag. ×1.5.

FIG. 2.—Tumor at site of injection of extract of primary human mammary carcinoma. Female. Mag. × 1.5.

Anal lesions occurred only in female animals. In the combined series totaling 129 females that received extracts derived from cancerous tissues, 36 developed anal lesions. In the series of 43 females that received the extracts of noncancerous tissues, only 2 developed the lesion.

Inquiry has revealed that this condition has been seen by others working with large colonies of mice, but since the author has been unable to find a description in the literature its inclusion in this report seems justified.

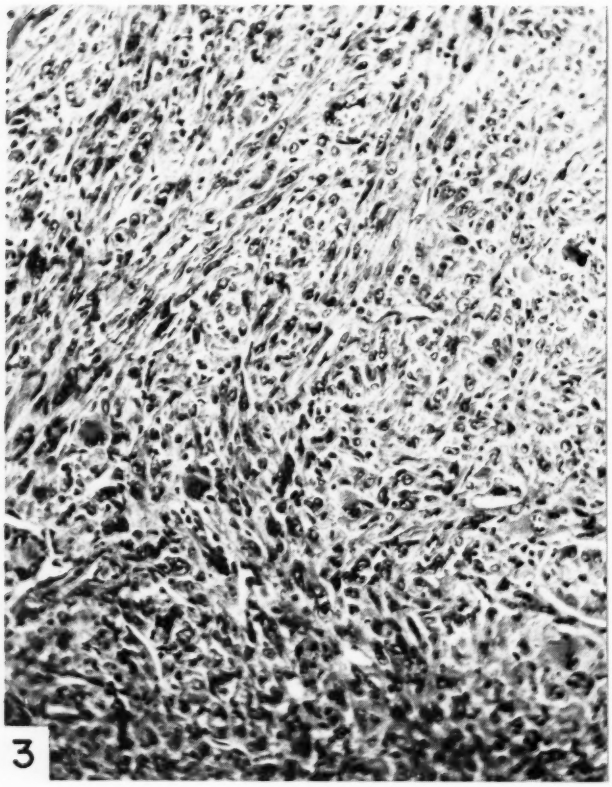
To consider this lesion a simple prolapse of the rectum would be to disregard the fact that the prolapse is preceded by a hyperplasia of the anal sebaceous glands. As it has not been seen previously by the author in animals kept under the same conditions, the incidence in these experiments suggests that some factor in the fat-soluble extracts employed may stimulate hypertrophy of the sebaceous glands. The prolapse appears to be the result of partial obstruction at the anal orifice.

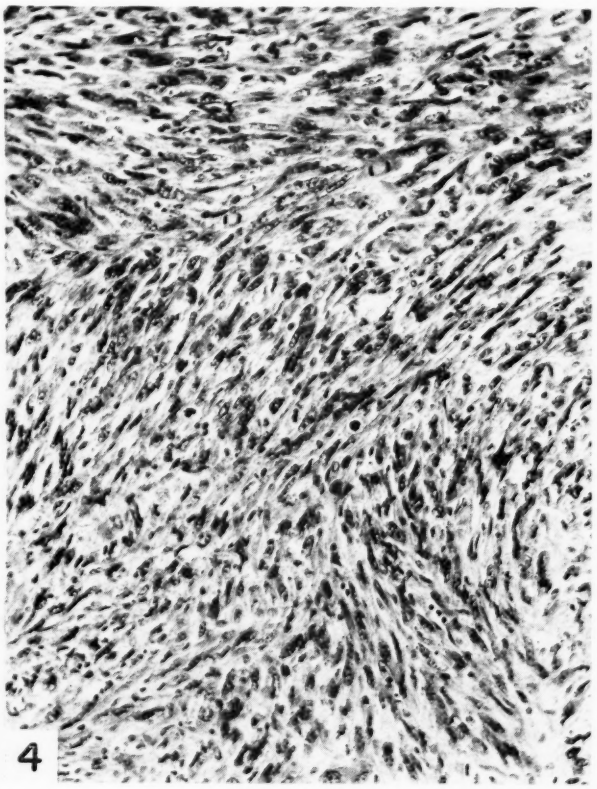
In the series of 40 animals treated with the nonsaponifiable fraction of the whole extracts of 3 cancerous breasts of types B and C, none developed tumors at the site of injection, but 10 developed anal lesions. The digitonide fraction of the nonsaponifiable matter

failed to induce tumors at the site of injection in 6 male animals. Of 6 females injected with the nondigitonide fraction, none developed tumors at the site of injection but 4 developed anal lesions. Injection of 18 females with the noncarbonyl fraction failed to produce tumors, but one animal developed an anal lesion.

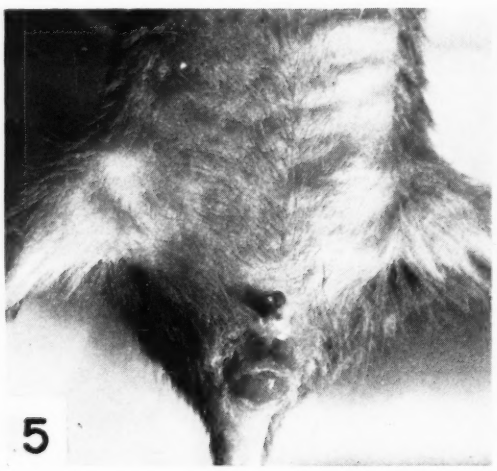
### Mammary Noncancerous Tissue

For a control experiment, similar extracts of 4 specimens of noncancerous mammary tissue have been tested (Table II). Among 54 animals treated with the whole extract, no tumors occurred at the site of injection. Two females developed anal lesions. Of 36 animals treated with the nonsaponifiable fraction of




Figs. 3 and 4.—Sections of the corresponding tumors shown in Figs. 1 and 2. Spindle cell sarcoma. Note numerous mitotic figures. Mag. × 161.



Figs. 5 and 6.—Animals showing early and late stages of anal lesions with prolapse. Mag. × 1.5.

the extracts from 3 of the specimens, no tumors developed at the site of injection, and none of the animals developed anal lesions.

### Rectal Cancerous Tissue

Extracts of 2 primary rectal carcinomas have been tested. These extracts were comparable to mammary cancer extracts of type C (Table III). In a series of 15 mice injected with the whole extracts, none has developed tumors at the site of injection; 2 anal lesions have occurred. In a series of 6 females treated with the nonsaponifiable fraction of one of the extracts, none developed tumors at the site of injection, but 3 developed anal lesions.

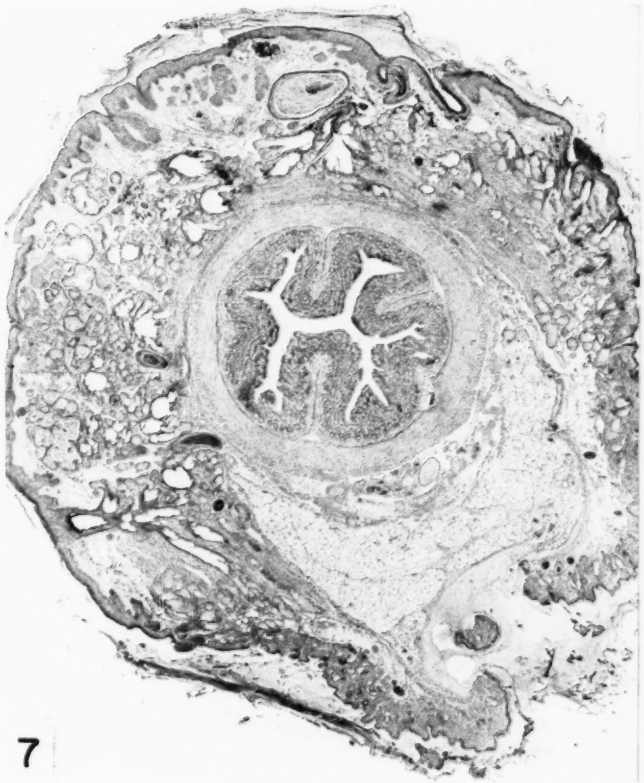


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Fig. 7.—Whole cross section taken at cutaneous junction of anal lesion. Mag. × 16.
Fig. 8.—Section showing hyperplastic anal glands. Mag. × 80.

Table II: Results with Noncancerous Mammary Tissue

| | Animals | | Injections | | Number developing tumors at site of injection | Number developing anal lesions | Animals still living | Duration of experiment, months |
|---|---|---|---|---|---|---|---|---|
| | Number | Sex | Number | Amount, cc. | | | | |
| WHOLE EXTRACT | | | | | | | | |
| H.N.Ca. #1 | 24 | 12♂ | 2 | 0.2 | 0 | 0 | 0 | 15 |
| | | 12♀ | 2 | 0.2 | 0 | 1 | 0 | 15 |
| H.N.Ca. #2 | 6 | 6♂ | 3 | 0.2 | 0 | 0 | 5 | 15 |
| H.N.Ca. #3 | 12 | 6♂ | 2 | 0.2 | 0 | 0 | 0 | 16 |
| | | 6♀ | 2 | 0.2 | 0 | 1 | 0 | 16 |
| H.N.Ca. #4 | 12 | 5♂ | 2 | 0.2 | 0 | 0 | 4 | 17 |
| | | 7♀ | 2 | 0.2 | 0 | 0 | 1 | 17 |
| NONSAPONIFIABLE FRACTION | | | | | | | | |
| H.N.Ca. #1 | 12 | 6♂ | 2 | 0.2 | 0 | 0 | 0 | 15 |
| | | 6♀ | 2 | 0.2 | 0 | 0 | 0 | 15 |
| H.N.Ca. #2 | 12 | 6♂ | 2 | 0.2 | 0 | 0 | 5 | 15 |
| | | 6♀ | 2 | 0.2 | 0 | 0 | 5 | 15 |
| H.N.Ca. #4 | 12 | 6♂ | 2 | 0.2 | 0 | 0 | 6 | 14 |
| | | 6♀ | 2 | 0.2 | 0 | 0 | 6 | 14 |

### Osteogenic Sarcoma

The whole extract of one osteogenic sarcoma of the femur has been tested in a series of 12 mice (Table IV). The test is now in the 13th month. One male animal developed an exceptionally malignant sarcoma (Fig. 9) at the site of injection in the left axillary region on the 249th day. Fig. 10 shows the histological picture. Microscopically, the growth does not appear greatly different from the other sarcomas reported here, but it is exceptional in that upon transplantation it becomes palpable 3 to 4 days earlier than the others and increases in size at about twice the rate. All tumors

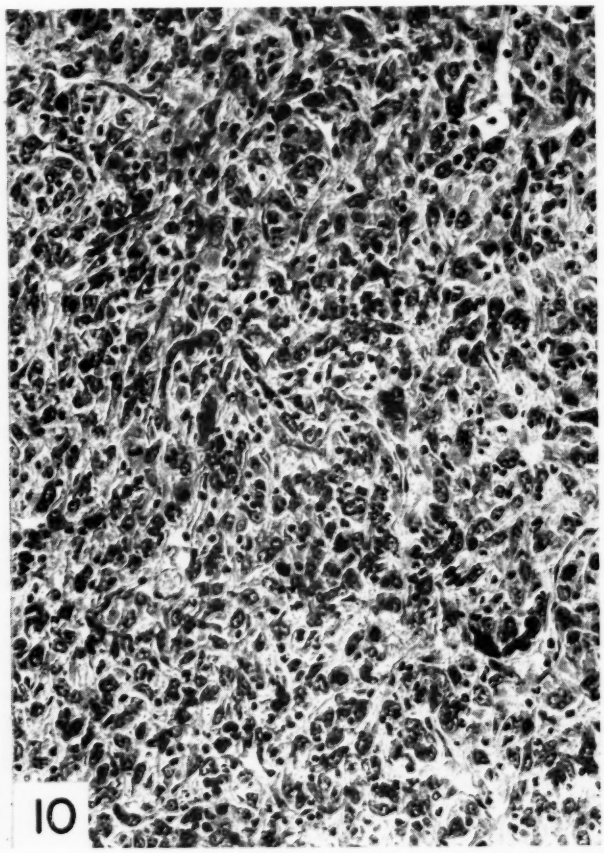

Fig. 9.—Tumor at site of injection of extract of human osteogenic sarcoma. The tumor is seen in the right lower quadrant of the abdomen of a male animal. Mag. × 1.

Fig. 10.—Section of the tumor shown in Fig. 9. Sarcoma. Mag. × 200.

Table III: Results with Cancerous Rectal Tissue

| | Animals | | Injections | | Number developing tumors at site of injection | Number developing anal lesions | Animals still living | Duration of experiment, months |
|---|---|---|---|---|---|---|---|---|
| | Number | Sex | Number | Amount, cc. | | | | |
| WHOLE EXTRACT | | | | | | | | |
| H.R.Ca. #1 | 9 | 4♂ | 3 | 0.2 | 0 | 0 | 0 | 19 |
| | | 5♀ | 3 | 0.2 | 0 | 2 | 0 | 19 |
| H.R.Ca. #2 | 6 | 6♂ | 3 | 0.2 | 0 | 0 | 0 | 15 |
| NONSAPONIFIABLE FRACTION | | | | | | | | |
| H.R.Ca. #2 | 6 | 6♀ | 3 | 0.2 | 0 | 3 | 0 | 17 |

Table IV: Results with Ostiogenic Sarcoma

| | Animals | | Injections | | Number developing tumors at site of injection | Number developing anal lesions | Animals still living | Duration of experiment, months |
|---|---|---|---|---|---|---|---|---|
| | Number | Sex | Number | Amount, cc. | | | | |
| WHOLE EXTRACT | | | | | | | | |
| H. Ost. Sarc. #1 | 12 | 6♂ | 2 | 0.3 | 1 | 0 | 5 | 13 |
| | | 6♀ | 2 | 0.3 | 0 | 0 | 6 | 13 |

reported here have yielded 100 per cent takes on transplantation, and 90 per cent or more of the animals have died of their tumors within 30 days following transplantation.

## DISCUSSION

One hesitates to discuss the inconclusive results of an investigation of this nature because of the risk of suggesting, unintentionally, that conclusions have been drawn.

The author recognizes the possibility that the occurrence of sarcomas in the animals treated with the whole extract of primary cancerous nodules may well be due to some nonspecific factor or factors. Sarcomas have resulted from the subcutaneous injection of many substances of a nonspecific nature. However, if for the purpose of discussion of these limited results the demonstrated factor is assumed to be of a specific nature, then a tentative suggestion can be made concerning its probable chemical nature.

Apparently the factor is not related to the carcinogenic hydrocarbons. The results suggest a more labile type of compound, susceptible to alteration when subjected to the conditions of alkaline hydrolysis at moderately high temperatures. If not, then the factor should have been demonstrable in the nonsaponifiable fraction of the extracts of mammary cancerous tissues of type C. It is unfortunate that the saponifiable fractions have not yet been tested. Had they also been found negative a definite conclusion as to the lability of the factor would be possible. Since those working with extracts of human liver have found that the carcinogenic activity is present in the nonsaponifiable fraction, it appears that the factor present in the whole extracts of primary mammary cancer is of a different nature.

The failure of the whole extracts of mammary cancerous tissue of type C to produce tumors at the site of injection suggests that the factor either is present only in the cancerous tissue of the primary nodule or is in greatest concentration there, and that that portion of the extract obtained from the surrounding uninvolved fat diluted the active factor from the cancerous nodule to an insufficient concentration.

Because of these results, the method of preparation has been changed for further experiments now under way. The extracts are now being made by a method in which room temperature is the maximum reached once the body heat has been removed by prompt freezing of the surgical specimen. The use of a nitrogen atmosphere is also being added to the method. Until the results of these further investigations have been obtained, conclusions are purposely being avoided.

## SUMMARY

1. In a series of 36 mice treated with extracts of three primary cancerous nodules obtained from surgically removed specimens of carcinomatous human breasts, 7 developed sarcomas at the site of injection.

2. In a series of 33 mice treated in a similar manner with an extract of a carcinomatous breast in which the primary cancerous nodule was absent, none developed a tumor at the site of injection.

3. In a series of 18 mice treated in the same way with extracts of two carcinomatous breasts in which the primary cancerous nodule was present, none developed a tumor at the site of injection.

4. In a series of 70 mice treated with the nonsaponifiable, digitonide, nondigitonide, and noncarbonyl fractions of the whole extracts of cancerous breasts, none developed a tumor at the site of injection.

5. In a series of 90 mice treated with the whole extract or the nonsaponifiable fraction of four noncancerous breasts, none developed a tumor at the site of injection.

6. In a series of 21 mice treated with the whole extracts or the nonsaponifiable fractions of two rectal carcinomas (type C), none developed a tumor at the site of injection.

7. In a series of 12 animals treated with the whole extract of one human osteogenic sarcoma, one animal developed a sarcoma at the site of injection.

8. In the combined series of 280 mice, 108 males and 172 females, 38 females developed a hyperplasia of the anal sebaceous glands and a prolapse of the rectum. Thirty-six of these anal lesions occurred among 129 females treated with the extracts of cancerous tissues. Two anal lesions occurred in the series of 43 females treated with the extracts of noncancerous tissues.

Conclusions are being deferred until further work has been completed.

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# The Effect of α-Estradiol-3-Benzoate on the Response of CHI Mice to 20-Methylcholanthrene

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(Received for publication March 23, 1942)

The role of estrogenic substances in the production of neoplasms of the male and female genital tracts and of the mammary glands of both sexes has been discussed at length by many authors. It appears that the conclusions reached depend largely upon a number of variables; among these are the criteria of malignancy chosen, the specific estrogen, the route of administration, and the animals themselves.

However, it would appear from the excellent review of Gardner (1) that the estrogens really have some effect on the production of mammary, uterine, vaginal, and testicular neoplasms. Factors which modify this relation between estrogen and the induction of neoplasms include the heredity of the experimental animal, the type of estrogenic substance used, and the duration of treatment.

In this connection it is interesting to note that some carcinogenic hydrocarbons are believed to have estrogenic properties (4).

Although these considerations might indicate the existence of some relationship between the sex glands and reactions to carcinogenic hydrocarbons, such has not been the case. Indeed, Stewart (8) has pointed out that castration seems to have no significant effect on the carcinogenic action of 1,2,5,6-dibenzanthracene in C3H mice.

Pierson (5) treated 2 rabbits for 13 months with folliculin and tar and noted that one developed a carcinoma and the other an adenoma of the uterus. Gilmour (2) used mixed stock mice and painted 3,4-benzpyrene and estrone on the skin. The compounds were dissolved in chloroform. She concluded that estrone increased the susceptibility of the skin to the carcinogen and that it also hastened the appearance of the tumors. Perry and Ginzton (3) also employed stock mice. They dissolved estrone and 1,2,5,6-dibenzanthracene in benzene and painted this mixture, and 1,2,5,6-dibenzanthracene alone, on the skin. There was a higher incidence of tumors in the group receiving estrone and the carcinogen than in the group receiving the carcinogen alone.

Recently Smith, Wells, and D'Amour (7) have injected rats with 20-methylcholanthrene in paraffin and with estradiol benzoate in sesame oil. They found that neither castration nor the estradiol benzoate affected the tumor incidence or the latent period of induction.

The present work was undertaken because it seemed desirable to study the possible cocarcinogenic effect of estrogens in a pure strain of mice. Furthermore it was believed that the results of Gilmour (2) and of Perry and Ginzton (3) might result in some measure from the toxic solvent they had used as a vehicle for the estrogen. Accordingly, the following experiment was undertaken.

## MATERIAL AND METHODS

All the mice used in this study were young adults of the CHI strain, approximately 3 months of age, obtained from Dr. L. C. Strong's colony at Yale University. At Dr. Strong's suggestion they were fed nurishmix pellets supplemented semiweekly with lettuce. They were housed in wooden boxes and fed from hoppers. The mice used for transplantation were offspring of additional animals obtained from Dr. Strong at the same time as the mice which were treated with estrogen and carcinogen.

The estrogen employed was estradiol benzoate [1] in sesame oil. The solution contained 1 mgm. per cc. and the animals were given subcutaneous injections of 0.05 cc. once weekly.

The carcinogen was 20-methylcholanthrene.[2] Under ether anesthesia 1.5 to 3.0 mgm. pellets were inserted subcutaneously in the dorsal region through a small incision, and the wound was closed with a single silk stitch.

The mice were observed daily, but tumors were measured and the dates of their appearance recorded at weekly intervals.

Transplants were made into young adult mice by injecting a saline suspension of tumor tissue subcutaneously with a trocar.

## RESULTS

As can be seen from Table I there was no essential difference in the latent period or in the incidence of tumors whether 20-methylcholanthrene was given alone or in combination with estradiol benzoate. All tumors arose subcutaneously in the region of the pellet.

The first occurred from 12 to 17 weeks after implantation of the pellets, when the mice were approximately 7 months old. The last tumor to appear was first palpable in the 24th week after pellet implantation, when the mice were about 9 months of age. The average latent period for the entire group was 18 weeks.

Histologically [3] all the growths except 2 mammary gland tumors in the untreated group were classed as

[1] α-Estradiol benzoate was generously supplied as progynon-B by Dr. Erwin Schwenk of the Schering Corporation.

[2] The 20-methylcholanthrene was prepared by Dr. W. E. Bachmann and supplied through the courtesy of Dr. J. T. Bradbury of the University of Michigan, Ann Arbor.

[3] I would like to thank Dr. Edgar H. Norris for his interest and for reviewing the microscopic classification of all the tumors.

sarcomas of grade II or III.[4] Four from animals treated with estrogen and carcinogen and 4 from animals treated with carcinogen only were transplanted through 4 transplant generations. In the first group 110 animals were used for hosts and 100 in the second. These sarcomas all grew at about the same rate, took in the same proportion of animals (78 and 82 per cent), and retained their histological identity. Those transplanted were used when they had reached 1.5 cm. in their greatest diameter. In all other cases animals with present experiment the co- or anticarcinogenic effect of estradiol would be shown only if it were very great, since large amounts of carcinogen were employed. However, it should be noted that where a cocarcinogenic effect of estrogen on the skin of the mouse has been reported large concentrations of carcinogen have been employed also. Our studies, like those of Smith, Wells, and D'Amour (7), show no cocarcinogenic effect of estrogens on the connective tissue, subject, of course, to the conditions of the experiment.

TABLE I: OCCURRENCE OF SARCOMAS IN CHI MICE TREATED WITH ESTROGEN AND CARCINOGEN

| Treatment | Sex | Total number | Number alive at 1st tumor | Number of tumors | Latent period, 1st tumor, weeks | Average latent period, weeks | Incidence * per cent |
|---|---|---|---|---|---|---|---|
| EB, MC | M | 25 | 17 | 14 | 15 | 17 | 82 |
| | F | 12 | 8 | 6 | 16 | 19 | 75 |
| | Both | 37 | 25 | 20 | 15 † | 18 | 80 |
| EB | M | 25 | — | 0 | — | — | 0 |
| | F | 12 | — | 0 | — | — | 0 |
| | Both | 37 | | 0 | | | |
| MC | M | 25 | 20 | 15 | 12 | 15 | 75 |
| | F | 12 | 9 | 5 | 17 | 20 | 55 |
| | Both | 37 | 29 | 20 | 14 † | 18 | 69 |
| None | M | 25 | — | 0 | — | — | 0 |
| | F | 12 | — | 0 | — | — | 0 |
| | Both | 37 | — | 0 | | | |

EB = 50γ α-estradiol-3-benzoate in 0.05 cc. sesame oil once weekly.
MC = Pellet of 20-methylcholanthrene (1.5 to 3.0 mgm.) subcutaneously.
* Percentage of animals, alive at time of appearance of 1st tumor, which developed tumors before death.
† Average of males and females.

tumors were allowed to live until they were dead or moribund. None of the growths metastasized, but practically all infiltrated through the body wall.

Two males, one treated with estradiol benzoate and a pellet of 20-methylcholanthrene and the other with only a pellet of 20-methylcholanthrene, developed what appeared in the gross to be neoplasms around the pellets, but on section they were classified as inflammatory reactions.

Characteristic estrogenic effects were seen in all animals which received estradiol benzoate. These included resorption of the pubic symphyses in the females, retention of urine and hydronephrosis, suppression of spermatogenesis, and pronounced stimulation of the female genital tract.

The cervices of all females were sectioned serially and studied. In no instance did they show changes which could be considered malignant.

## DISCUSSION

From the results reported by Shear and his associates (6) it is possible that under the conditions of the

[4] Tumors were graded histologically into four groups on the basis of differentiation and anaplasia.

## SUMMARY

α-Estradiol-3-benzoate had no effect on the incidence, degree of malignancy, or latent period of tumor induction by pellets of 20-methylcholanthrene in CHI mice.

The animals displayed the usual effects of hyperestrinism.

No malignant lesions of the cervix or upper vagina were observed in any of the animals.

I wish to thank Dr. Warren O. Nelson, Chairman of the Department of Anatomy, for encouragement and advice throughout this study and also for allowing me the use of his laboratory.

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# Phospholipids of Tumor Cells and Nuclei*

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(Received for publication, June 24, 1942)

The structure of the cell nucleus and its importance in the life of the cell have been studied extensively by the cytologist, but only recently has the biochemist begun to attack the problem of the chemical composition of the isolated cell nucleus. The investigator of tumor metabolism has a special interest in the cell nucleus since he deals with a continually proliferating tissue. Recent chemical studies on tumor nuclei consist of the work by Stoneburg (6) on the acetone-soluble and acetone-insoluble fractions of the lipid, and by Marshak (5) on the rate of turnover of the phospholipids. The following study was undertaken to determine the chemical nature of the phospholipid that is found in the tumor nucleus and to compare the nuclear phospholipid with that of the whole cell.

## EXPERIMENTAL

The tumor used throughout the experiment was carcinosarcoma 256. Healthy growing tissue from the periphery of tumors was dissected out and the necrotic central tissue discarded. A small weighed portion of this peripheral tissue was immediately extracted with lipid solvents (2, 3); the remainder was used for preparation of nuclei. For this purpose Stoneburg (6) employed 5 per cent citric acid followed by digestion with pepsin. Two per cent citric acid alone has been found adequate for isolation of nuclei from tumor. Treatment with ice-cold citric acid was followed by filtration through cheesecloth to remove the debris. The filtrate was centrifuged to bring down the nuclei. These were then washed with distilled water until the preparation was found to be completely free of cellular debris when examined with a microscope. After partial drying by repeated addition and evaporation of absolute alcohol, the preparation was thoroughly dried in a vacuum desiccator, weighed, and extracted with lipid solvents. Dry weights of whole tumor tissue were similarly obtained by macerating several weighed portions of tissue in a few cc. of 95 per cent alcohol and drying to constant weight in an oven at 105° C.

Phospholipids were obtained from the lipid extracts by removal of the solvent, extraction of the residue with petroleum ether, concentration of the extract, addition of dry acetone and magnesium chloride, and centrifugation. The phospholipid was dissolved in chloroform, made to volume, and aliquots were taken for determination of lipid phosphorus by the Kuttner and Lichtenstein method (4) and for choline by a modification (1) of the method of Beattie. Pooled samples of nuclear phospholipid were repeatedly tested for sphingomyelin by a method previously described (2).

## RESULTS AND DISCUSSION

Table I gives the phospholipid content of whole tissue and of corresponding nuclei. The percentage of choline and the *molar* ratio of choline to phosphorus in the phospholipid samples are also given in the table.

The phospholipid content of the whole tissue is approximately equal on a dry weight basis to that of the nuclei. Provided that nuclear constituents are not extracted during preparation of the nuclei, this type of comparison is valid. If water-soluble substances are removed by the citric acid, then the nuclear phospholipid content is actually lower than that of the whole tissue. Stoneburg's (6) values for nuclear phospholipid on the same tumor are slightly higher than those reported here. This discrepancy in results may result from a difference in method of preparation of nuclei, Stoneburg having used artificial gastric juice in addition to citric acid.

It can be seen from the table that the choline content of nuclear phospholipid is greater than that of whole tissue phospholipid. This indicates that in the nuclei there is a larger proportion of the choline-containing phospholipids, lecithin (and sphingomyelin), than in the whole cells.

A better index to the relative amounts of the choline-containing phospholipids is provided by the *molar* ratios of choline to phosphorus, since these are calculated without recourse to the use of the empirical factor of 25. Lecithin and sphingomyelin each has a ratio of 1.0. The presence of cephalin, which contains no choline, in a mixture of phospholipids lowers the ratio. Since the choline-to-phosphorus ratios as shown

* This study was supported by a grant from The International Cancer Research Foundation.

in the table are well below unity, the phospholipid of whole tumor cells and of their nuclei must contain considerable cephalin. The average ratio for nuclear phospholipid is 0.61, while that for the whole tissue phospholipid is 0.52. This difference between ratios is statistically significant. A higher proportion of lecithin and sphingomyelin in the nuclei than in the whole cells would account for this difference. On the basis of solubility, oxidative stability, and iodine numbers, Stoneburg (6) suggested that the nuclear phospholipids may be sphingomyelins. We have been unable to show the presence of sphingomyelin in the nuclear lipids except in trace amounts which may have been caused by slight cytoplasmic contamination. Thus with a 0.61 ratio and no sphingomyelin the nuclear phospholipid must be composed of about 60 per cent lecithin and 40 per cent cephalin, whereas it can be calculated from our previous data (1, 2) that the whole tissue phospholipid contains about 35 per cent lecithin, 45 per cent cephalin, and 20 per cent sphingomyelin (tumor dry residue = 16.2 per cent; molecular weights—lecithin = 777, cephalin = 723, sphingomyelin = 833).

It is evident from the choline-to-phosphorus ratios of 0.52 for whole tissue and 0.61 for nuclei that the ratio in the cytoplasm must be considerably lower than the 0.52 obtained for whole cells. The exact ratio for the cytoplasm depends on the relative proportions of cytoplasm and nuclei for this tumor. These proportions cannot be determined from the weights of dry nuclei and dry tissue since the nuclei are not quantitatively recovered from the tissue by the citric acid procedure.

TABLE I: THE PHOSPHOLIPIDS OF WHOLE TISSUE AND OF NUCLEI OF CARCINOSARCOMA 256

| Whole tissue phospholipid | | | Nuclei phospholipid | | |
|---|---|---|---|---|---|
| Gm.*/100 gm. of dry tissue | Choline content, per cent | Moles choline / Moles P | Gm.*/100 gm. of dry nuclei | Choline content, per cent | Moles choline / Moles P |
| 10.2 | 7.2 | 0.46 | 11.7 | 8.9 | 0.57 |
| 9.7 | 8.7 | 0.56 | 11.7 | 9.0 | 0.58 |
| 9.9 | 8.1 | 0.52 | — | — | — |
| 9.4 | 7.8 | 0.50 | 9.7 | 9.4 | 0.60 |
| 9.0 | 7.7 | 0.50 | — | — | — |
| — | — | — | 11.2 | 9.2 | 0.59 |
| 8.4 | 8.8 | 0.56 | 8.2 | 9.3 | 0.59 |
| 9.6 | 9.2 | 0.59 | 10.1 | 9.2 | 0.59 |
| 10.4 | 7.2 | 0.46 | 11.2 | 10.5 | 0.67 |
| 10.1 | 8.9 | 0.57 | 8.1 | 10.3 | 0.66 |
| 9.6 | 8.2 | 0.52 | 9.9 | 9.3 | 0.60 |
| — | — | — | 9.1 | 9.6 | 0.60 |
| — | — | — | 7.8 | 9.8 | 0.63 |
| — | — | — | 8.7 | — | — |
| — | — | — | 8.7 | 10.2 | 0.65 |
| Average † ..... 9.6 ± 0.56 | 8.2 ± 0.67 | 0.52 ± 0.04 | 9.7 ± 1.34 | 9.6 ± 0.51 | 0.61 ± 0.03 |

* Lipid P × 25 = phospholipid.

† In these averages the value following the ± sign is the standard deviation.

It is evident from the choline-to-phosphorus ratios of 0.52 for whole tissue and 0.61 for nuclei that the ratio in the cytoplasm must be considerably lower than the 0.52 obtained for whole cells. The exact ratio for the cytoplasm depends on the relative proportions of cytoplasm and nuclei for this tumor. These proportions cannot be determined from the weights of dry nuclei and dry tissue since the nuclei are not quantitatively recovered from the tissue by the citric acid procedure.

3. Since no sphingomyelin has been found in the nuclei, this phospholipid must be concentrated in the cytoplasm and a high proportion of lecithin probably accounts for the high choline content of the nuclei.

## SUMMARY

A comparison of the phospholipid from whole tissue of carcinosarcoma 256 with that from corresponding nuclei shows that:

1. The phospholipid content on a dry weight basis is essentially the same in both whole tissue and nuclei.

2. The molar ratios of choline to phosphorus (and thus the choline-containing phospholipids) are significantly higher in the nuclei.

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# Abstracts

## Reports of Experimental Research

### Carcinogenic Compounds

**BERENBLUM, I., and SCHOENTAL, R. [Sir William Dunn Sch. of Path., Oxford, England] THE QUANTITATIVE ESTIMATION OF 3:4-BENZPYRENE IN WHOLE ANIMALS, THEIR TISSUES AND EXCRETA. Biochem. J., 36:86-91. 1942.**

This method is sensitive to 0.02 to 0.05 µgm. benzpyrene and is accurate to ±20% over a range of 1 to 10,000 µgm. per mouse; losses in the process are of the order of 5%. A mouse, or tissues, are saponified with alcoholic KOH and after acidification with concentrated HCl are extracted with benzene. The benzene layer after washing is extracted with $Na_2CO_3$ solution and centrifuged. Sometimes, when the soap content is low, stearic acid is purposely added, as emulsification (resolved by centrifuging) is found to be beneficial to purification. The benzene layer (now colorless) is dried and passed through alumina (differential adsorption from benzene-petroleum ether is sometimes necessary). By fluorescence spectroscopy, the bands are photographed on the same plate as those of standard solutions of benzpyrene and matching is made by eye.—I. H.

**BERENBLUM, I., and SCHOENTAL, R. [Sir William Dunn Sch. of Path., Oxford, England] THE RATE OF DISAPPEARANCE OF 3:4-BENZPYRENE FROM THE MOUSE AFTER SUBCUTANEOUS AND INTRAPERITONEAL INJECTION. Biochem. J., 36:92-97. 1942.**

Experiments by other workers have suggested that the carcinogenicity of a hydrocarbon is related to its persistence at the site of injection. Groups of mice were given a single injection with exact amounts of benzpyrene (20 µgm., 200 µgm., 2 mgm., 10 mgm.) in sesame oil, and the animals were killed at intervals for assay of hydrocarbon. The benzpyrene disappears from the body 15 times more quickly if the injection is made intraperitoneally than if it is made subcutaneously. Logarithmic curves of the rate of disappearance show that this is proportional to the concentration of hydrocarbon, suggesting that diffusion is the limiting factor. After 8 months, intraperitoneally injected mice developed no tumors but several growths had appeared in the subcutaneously injected series, even in the 20 µgm. group. The authors conclude that as persistence of the hydrocarbon favors tumor formation, the agent is the unchanged benzpyrene rather than derived products.

Observation of the injection sites in ultraviolet light after all benzpyrene disappeared showed the persistence of a blue fluorescing substance proved to be a contaminant of the benzpyrene used. Benzpyrene could be detected in the blood of a mouse one hour after injection of 10 mgm. of the hydrocarbon; and the benzpyrene content of blood, spleen, kidney, lungs, brain, liver, and gall bladder, respectively, were similar 9 days and 33 days after injection, showing that no accumulation had taken place.

Mice injected with 10 mgm. of benzpyrene excrete in the feces 0.35 to 0.6% of the amount which has disappeared from the body; thus less than 1% can be acounted for as excreted unchanged.—I. H.

**BERENBLUM, I., and SCHOENTAL, R. [Sir William Dunn Sch. of Path., Oxford, England] METABOLIC PRODUCTS OF 3:4-BENZPYRENE. Nature, London, 149:439-440. 1942.**

Benzpyrene is metabolized about 15 times more rapidly when injected intraperitoneally than when injected subcutaneously. Dried feces from rats previously injected intraperitoneally with large amount of benzpyrene were extracted with cold benzene. Purification of the benzene extract led to the isolation of a phenolic substance similar to BPX (*cf.* Chalmers, J. G., and Crowfoot, D., Biochem. J., **35**:1270. 1941) and of a red quinone apparently identical with 3,4-benzpyrene-5,8-quinone. Reductive acetylation or methylation of the quinone gives products which differ from acetyl or methyl derivatives of BPX. This is in agreement with the conclusion of Chalmers and Crowfoot that BPX is a monohydroxy derivative of benzpyrene. On the other hand on standing in air or by the action of oxidizing agents BPX is converted into the red quinone which suggests that BPX might be a dihydroxy derivative.

It is suggested that metabolism of benzpyrene in the animal body leads to the formation of both a mono- and a dihydroxy-benzpyrene, the former being slowly converted into the latter, and the latter being rapidly converted into a quinone.—F. L. W.

**BONSER, G. M. [Univ. of Leeds, England] MALIGNANT TUMOURS OF THE INTERSTITIAL CELLS OF THE TESTIS IN STRONG A MICE TREATED WITH TRIPHENYLETHYLENE. J. Path. & Bact., 54:149-154. 1942.**

Ten Strong A males received weekly, from the age of 4 weeks, 3 mgm. of triphenylethylene in sesame oil by subcutaneous injection. Of 8 mice surviving 50 weeks of treatment, all developed bilateral interstitial cell tumors of the testes; in 5 mice both tumors were malignant, in 2 there were a malignant and a simple tumor. Microscopically the tumors were similar to those in a previous series. Of the total of 16, 2 were classified as adenomas and the remainder as carcinomas. There were metastatic deposits in 2 mice treated for 67 and 75 weeks respectively; dissemination took place *via* the lymphatic vessels in one case, and primarily by the blood stream in the other. One of these animals also developed a mammary

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carcinoma. The occurrence of metastasis was related to the age of the tumors in the testis: in 6 mice without metastases the average duration of the malignant tumors was 12.5 weeks (7 to 22 weeks), whereas in the 2 mice with metastases the duration was 28 and 30 weeks. Attempts were made to graft from 3 of the primary malignant tumors to the testes, peritoneum, and subcutaneous tissues of 11 closely related mice of the same strain, but with negative results. The paper is illustrated with 6 photomicrographs.—A. H.

**DICKENS, F., and WEIL-MALHERBE, H. [Royal Victoria Infirmary, Newcastle-upon-Tyne, England] INVESTIGATION INTO THE POSSIBLE CARCINOGENIC ACTIVITY OF WOOD SMOKE. Cancer Research, 2:680-684. 1942.**

Four groups of 20 mice were painted twice a week for a period up to 24 months with 4 fractions of wood smoke condensates prepared under conditions closely resembling those employed in the food-smoking industry. At the site of application only one papilloma was observed which appeared after 22 months and spontaneously regressed a month later. On the other hand, single and multiple pulmonary adenomas were found in 8 of 17 mice painted for 18 months or more with the tar fraction which had settled out from the "pyroligneous liquor."

In another series of experiments rats were fed smoked fish or meat 3 times weekly for periods up to 20 months. No neoplasms developed.—Authors' summary.

**DUNLAP, C. E., and WARREN, S. [Harvard Med. Sch., Boston, Mass.] THE INCIDENCE OF PRIMARY LUNG TUMORS IN MICE WITH INDUCED SARCOMAS. Cancer Research, 2:685-687. 1942.**

Autopsies were performed on 1,705 C3H mice injected subcutaneously or intraperitoneally with various carcinogenic and noncarcinogenic hydrocarbon derivatives. Sarcomas were readily induced at the site of injection but the incidence of pulmonary and hepatic adenomas was very low.

With the same compounds 1,024 pure strain Swiss mice were similarly treated. Carcinogenic agents increased the incidence and accelerated the rate of appearance of primary lung tumors. The incidence of primary lung tumors was several times higher in those mice developing tumors at the site of injection of a carcinogenic agent than it was in mice treated identically but failing to develop such tumors. This result indicates that, within an inbred strain, those mice most susceptible to the induction of subcutaneous and intraperitoneal tumors are also more susceptible to the induction of primary lung tumors.—Authors' abstract.

**WARREN, F. L. [Chester Beatty Research Inst., Royal Cancer Hosp. (Free) London] AEROBIC OXIDATION OF AROMATIC HYDROCARBONS IN THE PRESENCE OF ASCORBIC ACID. Chem. & Ind., 61:170. 1942.**

Aromatic hydrocarbons in aqueous acetone solutions containing ascorbic acid are oxidized in the presence of air. The oxidation takes place in the dark. Oxidation of naphthalene, phenanthrene, anthracene, and the carcinogenic hydrocarbons 3,4-benzpyrene, cholanthrene, and methylcholanthrene was observed.

Anthraquinone was identified in the oxidation products from anthracene. The oxidation of 3,4-benzpyrene leads to the formation of a mixture of the two known quinones (5,8- and 5,10-), together with a phenolic substance. The latter dissolves in alkali to give a solution with a strong green fluorescence, and is thus similar to the phenol isolated as a metabolic product of benzpyrene (BPX).

The oxidations also occur if dihydroxymaleic acid is substituted for ascorbic acid. This fact, together with the observation that copper inhibits the oxidation, indicates that the enediol grouping of ascorbic acid is the essential structure necessary for the reaction.

The possibility that ascorbic acid plays a part in *in vivo* detoxication of aromatic hydrocarbons is considered.—F. L. W.

## Hormones

**BITTNER, J. J. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] POSSIBLE RELATIONSHIP OF THE ESTROGENIC HORMONES, GENETIC SUSCEPTIBILITY, AND MILK INFLUENCE IN THE PRODUCTION OF MAMMARY CANCER IN MICE. Cancer Research, 2:710-721. 1942.**

Abstracted in the Report of the Endocrine-Cancer Conference, Cancer Research, 2: 723. 1942.

**SPEERT, H. [Johns Hopkins Hosp., Baltimore, Md.] "PALE EPITHELIUM" IN THE MAMMARY GLAND AND ITS EXPERIMENTAL PRODUCTION IN THE RHESUS MONKEY. Surg., Gynec. & Obst., 74:1098-1105. 1942.**

Changes resembling this condition have been produced in the mammary glands of rhesus monkeys by the administration of very large amounts of estrogenic hormones, especially in castrated animals. Pale epithelium represents a metaplastic alteration of normal mammary gland cells, but no support is offered for the belief that it has any significance for the development of carcinoma.—H. G. W.

## Genetics

**MILLER, E. W., and PYBUS, F. C. [Royal Victoria Infirmary, Newcastle-upon-Tyne, England] THE EFFECT OF OESTRONE ON MICE OF THREE INBRED STRAINS, WITH SPECIAL REFERENCE TO THE MAMMARY GLANDS. J. Path. & Bact., 54:155-168. 1942.**

Mice of three inbred strains differing in their incidence of spontaneous mammary carcinoma were divided into four classes in each strain—ovariectomized and nonovariectomized females, castrated and noncastrated males—and given weekly subcutaneous injections of 300 units of ketohydroxyestrin from the age of about 5 weeks until death. This treatment produced 44 mammary tumors in 135 Simpson mice, 3 in 75 Edinburgh mice, and none in 112 CBA mice. The mammary glands of ovariectomized females responded more slowly and to a lesser degree than those of nonovariectomized females. Ovariectomy prolonged life. The mammary glands of castrated males responded more rapidly and to a greater degree than those of noncastrated males, and to a degree similar to that found in ovariectomized females. Castration shortened life. Treatment also produced 30 uterine tumors (mainly fibromas or fibrosarcomas of the cervix) in 54 CBA females; only 4 tumors of this type occurred in the Edinburgh and 2 in the Simpson strain. Treatment did not affect the incidence of lung tumors but greatly increased the incidence of various lymphadenopathies.—A. H.

## Biochemistry and Nutrition

**ANTOPOL, W., and UNNA, K. [Merck Inst. for Therapeutic Research, Rahway, N. J., and Newark Beth Israel Hosp., Newark, N. J.] THE EFFECT OF RIBOFLAVIN ON THE LIVER CHANGES PRODUCED IN RATS BY p-DIMETHYLAMINOAZOBENZENE. Cancer Research, 2: 694-696. 1942.**

Feeding of *p*-dimethylaminoazobenzene produced considerable hepatic damage in young rats maintained on a purified diet consisting of vitamin-free casein, dextrose, crisco, salts, and cod liver oil and supplemented with thiamin, pyridoxine, pantothenic acid, and choline. A nicotinic acid supplement was ineffective in preventing the pathologic changes in the liver.

The administration of 10 mgm. of riboflavin 3 times a week protected all animals from liver damage for a period of at least 4 months. However, supplementing continuously with riboflavin failed to prevent liver damage when the animals were exposed to *p*-dimethylaminoazobenzene for more than 5 or 6 months. Thereafter, histologic examination revealed severe involvement of the liver.—Authors' abstract.

**JONES, J. L. [Univ. of Missouri, Columbia, Mo.] THE EFFECT OF VITAMIN $B_1$ DEPRIVATION ON THE APPEARANCE, GROWTH RATE, AND COURSE OF THE JENSEN RAT SARCOMA. Cancer Research, 2:697-703. 1942.**

The effect of vitamin $B_1$ deprivation on the appearance, growth rate, and course of the Jensen rat sarcoma was studied at three stages of deprivation: (1) deprivation started on appearance of tumor, *i.e.*, 12 days after transplantation, (2) deprivation started on the day of transplantation, (3) deprivation started 12 days before transplantation with return to a normal diet 25 days after transplantation. $B_1$ deprivation, quite apart from caloric restriction, affected the course of the tumor in the following manner: Tumor growth rates were not affected by deprivation started 12 days after transplantation. This was accounted for by the fact that the tumor destroyed the animals before the deprivation had had any serious effect. Deprivation started on the day of transplantation slowed the tumor growth rate and thereby increased the survival period of the host. Deprivation started 12 days before transplantation resulted in a still later appearance of the tumors; it also further decreased the growth rate and size of the tumor, thus increasing the survival period still further. The return of $B_1$-free tumor-bearing rats to a normal diet after the 25th day of transplantation temporarily increased the tumor growth rate together with the body weight, but that $B_1$ deprivation had an effect on the subsequent course of the tumor is shown by the later appearance and by regressions even after the return of the host to a normal diet. $B_1$ deprivation, quite apart from caloric restriction, affected the course of the tumor.—Author's abstract.

**POTTER, V. R. [Univ. of Wisconsin, Madison, Wis.] THE INHIBITION OF SULFHYDRYL-CONTAINING ENZYMES BY SPLIT PRODUCTS OF p-DIMETHYLAMINOAZOBENZENE. Cancer Research, 2:688-693. 1942.**

Since the toxicity of the split products of *p*-dimethylaminoazobenzene for yeast zymase and succinoxidase is believed to be caused by interaction with sulfhydryl groups, the author selected a much simpler sulfhydryl-containing enzyme system (urease) in order to study the nature of the inhibition and to gain further support for the idea that sulfhydryl groups are involved in the case of the zymase and succinoxidase systems. Evidence that the toxicity is referable to the oxidized form of the split products is given, and it is shown that the toxicity is prevented but not reversed by cysteine. The mechanism of the inhibition is discussed, and direct combination of the enzyme sulfhydryl group and the oxidized split product is considered the most probable explanation. The inhibition of the other two sulfhydryl groups according to this mechanism is then discussed from the standpoint of *in vivo* conditions and of the concept that enzymes which are essential to normal tissue but not to cancer tissue should be inhibited by carcinogenic agents.—Author's abstract.

**WALDSCHMIDT-LEITZ, E., HATSCHEK, R., and HAUSMANN, R. [Deutsch. Karls-Univ., Prague, Czechoslovakia] ÜBER d-PEPTIDASE IM SERUM. [d-PEPTIDASE IN SERUM] Hoppe Seyler's Ztschr. f. physiol. Chem., 267:79-90. 1940.**

A number of observations are cited on the occurrence of *d*-peptidase in the serum of cancer patients and of rats bearing the Walker cancer. This finding is ascribed by the authors to some theoretical defensive mechanism. As substrate for the identification of *d*-peptidase the polypeptide *d*-leucyl-glycyl-glycine is employed. Intravenous injection of the racemic peptide into normal animals likewise gives rise to the corresponding enzyme in the blood serum, while similar treatment of the cancerous organism results in the disappearance of the previously present abnormal ferment. The paper also gives fragmentary data to support a hypothesis that injection of racemic peptide, by stimulation of the reticulo-endothelial system, may inhibit the carcinogenic potency of benzpyrene on the skin of the mouse. The longest periods of survival among 16 treated and 18 control animals were 40 and 45 days respectively, and papillomas developed in 8 mice of the latter group only.—M. J. E.

## Immunology

**CHEEVER, F. S., and MORGAN, H. R. [Harvard Med. Sch., Boston, Mass.] MECHANISM OF TUMOR IMMUNITY AS INVESTIGATED BY MEANS OF THE INTRAOCULAR INOCULATION OF THE BROWN-PEARCE CARCINOMA. Cancer Research, 2:675-679. 1942.**

In a study of implants of the Brown-Pearce carcinoma into the anterior chamber of the eyes of rabbits, a high percentage of takes was obtained in normal animals. Secondary intraocular inoculation in animals immunized by any one of several standard methods was unsuccessful in 18 of 20 cases. Secondary intraocular inoculations as long as 4 months after the primary implantation of tumor into the other eye were usually successful, regardless of the state of activity of the first growth at this time. Of 11 tumor fragments remaining as long as 14 days in the eyes of immune animals only 2 proved viable and capable of delayed but sustained proliferation when implanted into the eyes of normal animals.—Authors' abstract.

**GREENE, H. S. N. [Rockefeller Inst. for Med. Research, Princeton, N. J., and Yale Univ. Sch. of Med., New Haven,**

**Conn.] THE PARTICIPATION OF THE ANTERIOR CHAMBER OF THE EYE IN RESISTANCE PHENOMENA RELATED TO TUMOR GROWTH. Cancer Research, 2:669-675. 1942.**

A series of experiments was performed in an attempt to ascertain whether or not a blood-aqueous humor barrier separated the anterior chamber of the eye from the remainder of the body, and thus determined the ease with which tumor transplantation was effected at this site. It was found that, following tumor growth in the anterior chamber of one eye, both the testicle and the anterior chamber of the opposite side were resistant to reinoculation. On this basis it was concluded that the aqueous humor was not an isolated fluid but participated in general body reactions, and that its susceptibility to tumor transfer was not determined by an antibody barrier.

An interpretation of other reinoculation experiments based on this conclusion suggested that the resistance-provoking powers of the tumors used were directly related to their growth rates and longevity. The ability of the various tumors to grow in partially resistant hosts appeared, on the other hand, to be a function of their stromal requirements.—Author's abstract.

### COMPARATIVE ONCOLOGY

**BLOOM, F. [Flushing, N. Y.] SPONTANEOUS SOLITARY AND MULTIPLE MAST CELL TUMORS ("MASTOCYTOMA") IN DOGS. Arch. Path., 33:661-676. 1942.**

Neoplasms of the skin are very frequent in dogs, and in a group of 49 primary cutaneous tumors the author has encountered 5 of which the histologic structure consisted essentially of somewhat atypical tissue or histogenous mast cells with cytoplasm containing numerous metachromatic basophilic granules. Three of the tumors were solitary and benign and 2 were multiple and apparently malignant. With only 4 possible exceptions no neoplasms of a similar type have been reported in the human or veterinary literature. The cytoplasm of the neoplastic mast cells contains, in addition to the metachromatic basophilic granules, rod-shaped crystalloids, spherical basophilic bodies of solid or granular structure, and spherical acidophilic bodies. —H. G. W.

**STEINER, P. E., KLÜVER, H., and BRUNSCHWIG, A. [Univ. of Chicago, Chicago, Ill.] THREE CARCINOMAS OF THE TONGUE IN TWO MONKEYS. Cancer Research, 2: 704-709. 1942.**

Three squamous cell carcinomas of the tongue occurred simultaneously in two macaque monkeys (*Macaca mulatta* and *Macaca irus*) in a primate colony. One monkey died of aspiration pneumonia with local tumor metastases, and the other died after operation, without metastases. Attempts at transmission were made but results remain negative after 3½ months. None of the commonly quoted etiological factors for cancer of the tongue in man were present except that both animals were elderly males. Both had experimental brain lesions of long standing. This is the first report of cancer of the tongue in subhuman primates, and also the first in nondomesticated animals.

An addendum refers to a case of carcinoma of the tongue in a horse (Henson, J. Am. Vet. M. A., **94**: 124. 1939), and to a third instance of carcinoma of the tongue in a macaque.—Authors' abstract.

## Clinical and Pathological Reports

### ETIOLOGY

**CRAMER, W. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] THE ORIGIN OF CANCER IN MAN. J. A. M. A., 119:309-316. 1942.**

An excellent lecture discussing the etiology of cancer in the light of modern research. From a correlation of the results obtained by the study of experimental carcinogenesis in animals, with clinical and pathological observations, and with statistical investigations on cancer in man, it has become evident that there is a multiplicity of etiologic factors and that the etiology of cancer in man, as in animals, has to be considered a separate problem for each organ. We are entitled now to consider the possibility of preventing several forms of cancer in man by attacking the disease at its origin instead of remaining on the defensive until the disease has attacked man. This article should be read by all interested in cancer from any standpoint.—H. G. W.

### RADIATION—DIAGNOSIS AND THERAPY

**CASE, J. T. [Northwestern Univ. Med. Sch., Chicago, Ill.] A CLINICAL APPROACH TO THE ROENTGEN DIAGNOSIS OF CARCINOMA OF THE COLON. Illinois M. J., 80:145-152. 1941.**

An extensive review of the clinical and roentgenographic features of cancer of the colon is presented.—M. J. E.

**DONOVAN, M. S., and WARREN, S. [New England Deaconess Hosp., Boston, Mass.] PERSISTENCE OF TUMOR AFTER PREOPERATIVE RADIUM TREATMENT FOR CANCER OF THE CORPUS UTERI. Surg., Gynec. & Obst., 74: 1106-1111. 1942.**

A review of 46 proved cases of carcinoma of the uterine body, treated by uterine radon and complete hysterectomy at least 6 weeks after irradiation. In only 5 was the uterus, when removed, found to be free from carcinoma. This investigation does not throw light on the question of the value of radiation in preventing vaginal metastasis. In deciding whether or not to use preoperative radiation, the relative advantages of possibly destroying the tumor or decreasing its chances of implantation must be balanced against the delay and inconvenience to the patient.—H. G. W.

**HOLMES, G. W. [Harvard Med. Sch., Boston, Mass.] THE PRESENT STATUS OF IRRADIATION TREATMENT IN NEOPLASTIC DISEASE. Texas State J. Med., 37:481-486. 1941.**

A general review.—M. J. E.

**WIGBY, P. E. [Houston, Tex.] INTRA-ORAL RADIATION THERAPY. Texas State J. Med., 37:422-428. 1941.**

Of 18 patients with tumors in the mouth, treated with roentgen radiation, 6 were symptom-free after 1 to 3 years, and 12 died. Photographs illustrate the technic employed.—M. J. E.

## Skin and Subcutaneous Tissues

**BROWN, J. B. and McDOWELL, F. [Washington Univ. Sch. of Med., St. Louis, Mo.] "FIELD-FIRE" AND INVASIVE BASAL CELL CARCINOMA—BASAL-SQUAMOUS TYPE. Surg., Gynec. & Obst., 74:1123-1132. 1942.**

The term "field-fire" is applied to basal cell carcinomas which have pronounced superficial invasive properties, and often a center that appears free from involvement. Microscopically this tumor shows an advancing edge of basal cell carcinoma, while in the center there is left scarred derma and scar epithelium. Invasive basal cell carcinoma is a clinical type which once firmly established is practically incurable with the means now at our command. It may recur years after operative removal. Basal cell carcinomas are definitely more dangerous than is generally thought and taught.—H. G. W.

**WOOLHANDLER, H. W., and BECKER, S. W. [Univ. of Chicago Sch. of Med., Chicago, Ill.] ADENOMA OF SEBACEOUS GLANDS (ADENOMA SEBACEUM). WITH CONSIDERATION OF KERATOTIC ADENOMA SEBACEUM AND TRUE ADENOMA OF SEBACEOUS GLANDS. Arch. Dermat. & Syph., 45:734-756. 1942.**

Analysis is made of 28 cases of various types of so called adenomas of the sebaceous glands. Although a majority of the members of this group are not true adenomas, the designations are convenient in view of the uncertainty which exists concerning the use of the term "adenoma." The disease may be divided into 7 groups: adenoma sebaceum (Balzer), adenoma sebaceum (Pringle), acquired (senile) adenoma, keratotic adenoma sebaceum, sebaceous gland adenoma, nevus sebaceus, and nevus pigmentosus et sebaceus.—H. G. W.

## Nervous System

**HARTMAN, H., and HELFER, L. M. [San Antonio, Tex.] METASTATIC TUMORS INVOLVING THE CENTRAL NERVOUS SYSTEM. Texas State J. Med., 37:587-592. 1942.**

Eight cases of involvement of the brain or spinal cord by metastases are recorded. Clinically the process did not differ from that associated with primary growths of the nervous system. The presence, in 5 patients, of melanoma secondary to untreated or inadequately treated tumors of the skin, was ascertained by operation or necropsy. In 1 case, a metastatic hypernephroma in the brain was extirpated under the assumption that it was the primary neoplasm since evidence of a new growth in the kidney was not present. One patient had a mediastinal neoplasm extending into the dorsal vertebrae, and another, multiple foci of invasion of the spine, skull, and cervical lymph nodes, by a Ewing's sarcoma.—M. J. E.

**RITTER, S. A. [Misericordia Hosp., New York, N. Y.] PRIMARY NEUROGENIC SARCOMA OF THE FOREARM FOLLOWING TRAUMA AT GOLF. CASE REPORT. Am. J. Surg., 56:501-503. 1942.**

The tumor arose at the site of a trauma received but 5 weeks before. It was removed, and there has been no recurrence in 2½ years.—H. G. W.

**WALKER, A. E. [Univ. of Chicago, Chicago, Ill.] EARLY DIAGNOSIS OF BRAIN TUMORS. Illinois M. J., 80:286-292. 1941.**

While such classical manifestations as headache, vomiting, and choked discs have always been considered to be associated with cerebral neoplasms, they are now known to be late effects of the disease, dependent upon an increase in intracranial pressure. The general nature of the initial symptom complex is subject to variation because of differences in the structure of the tumors or in their location. Even when a growth does not produce a sudden onset of alarming symptoms, eventually some steadily increasing dysfunction of the nervous system will be induced. Abnormalities in the electroencephalographic records may assist in the localization of approximately 50% of the tumors. The usual roentgen studies frequently offer corroborative evidence, but it may be necessary to resort to encephalography or ventriculography, especially in those patients without papilledema. Arteriographic investigations are not as yet sufficiently standardized to permit an estimate of their possible ultimate value.—M. J. E.

## Eye

**AVERY, J. W., and WARREN, J. W. [Hollywood, Calif.] AN UNUSUAL CASE OF HODGKIN'S DISEASE. A PRELIMINARY REPORT. Arch. Ophth., 26:1019-1022. 1941.**

Involvement of the eye by Hodgkin's disease is unusual. In the case recorded the patient had evidence of generalized lymphadenopathy. The diagnosis was made by biopsy. Bilateral masses in the outer aspects of the eyeballs were presumptively of the same nature. The general and ocular lesions regressed following roentgen therapy, and the patient appeared well 2 years later.—M. J.E.

**KLIEN, B. A. [Rush Med. Coll., Univ. of Chicago, Chicago, Ill.] DETACHMENT OF THE PARS CILIARIS RETINAE. A CONTRIBUTION TO THE DIAGNOSIS OF MALIGNANT INTRAOCULAR TUMORS. Arch. Ophth., 26:347-357. 1941.**

Detachment of the pars optica of the retina occurs in a variety of conditions, but when combined with involvement of the pars ciliaris, the presumptive diagnosis ophthalmoscopically is malignant intraocular tumor. These findings, dependent upon an increase in subretinal pressure, are, therefore, of singular value in the early diagnosis of tumors of the eye. Illustrations are included.—M. J. E.

**ROTTINO, A., and KELLY, A. S. [St. Vincent's Hosp., New York, N. Y.] SPECIFIC NERVE SHEATH TUMOR OF ORBIT (NEURILEMMOMA, NEURINOMA). REPORT OF A CASE WITH REVIEW OF LITERATURE. Arch. Ophth., 26:478-488. 1941.**

A neurinoma of characteristic morphology was successfully excised from an eyeless orbit. The eye had been removed 20 years previously because of the existence of a neoplasm, the nature of which cannot now be ascertained.—M. J. E.

**SAMUELS, B. [New York, N. Y.] TUMORS OF CONJUNCTIVA AND LIDS. A BRIEF REVIEW. Arch. Ophth., 26:789-796. 1941.**

The clinical aspects and the treatment of these neoplasms are reviewed.—M. J. E.

**STOUGH, J. T. [Houston, Tex.] MELANOSARCOMATA OF THE LID MARGIN AND CONJUNCTIVA: WITH CASE REPORTS. Texas State J. Med., 37:675-679. 1942.**

Two cases of melanoma developing in pigmented zones present in the eye since birth are recorded. In the first, melanoma of the ocular conjunctiva was treated success-

fully by means of 2 extensive local resections and the patient appeared cured after 5 years, while in the second, distant metastases from a growth on the lower lid developed rapidly despite exenteration of the orbital tissues. —M. J. E.

### Breast

**WAX, W. V. [Mem. Hosp. of Greene County, Catskill, N. Y.]. ULCERATING CANCER OF BREAST AS A PORTAL OF ENTRY FOR FATAL TETANUS. Am. J. Surg., 56:680-683. 1942.**

Report of a unique case.—H. G. W.

**YOUNG, T. O., and PARSON, E. I. [Duluth and Askov, Minn.] PATHOLOGICAL SIGNIFICANCE OF DISCHARGE FROM THE NON-LACTATING BREAST. Journal-Lancet, 61: 446-450. 1941.**

Abnormal discharge from the breast, while almost always a sign of a lesion in the organ, is not necessarily indicative of malignant tumor. Of 468 patients with mammary disease, 40 (8.5%) had some form of discharge, which usually was serosanguinous or frankly hemorrhagic. Nevertheless, the pathologic state in 13 patients was subsequently found to be a chronic cystic mastitis, in 12 a papilloma, and in 15 a cancer. No relationship appeared to be discernible between the type of discharge and the existing lesion.—M. J. E.

### Female Genital Tract

**COUNSELLER, V. S. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE UTERUS. Surg. Clin. North Am., 21: 1189-1199. 1941.**

This review is concerned with the following aspects of carcinoma of the uterus: etiology, site, degree of malignancy, extent of growth, symptoms, age distribution, diagnosis, and treatment.—J. L. M.

**DEIBERT, A. V., and GREENBLATT, R. B. [U. S. Public Health Service Med. Center, Hot Springs, Ark., and Univ. of Georgia Sch. of Med., Augusta, Ga.] MALIGNANCY AND LYMPHOGRANULOMA VENEREUM. Am. J. Syph., Gonor. & Ven. Dis., 26:330-335. 1942.**

A case is reported in which lymphogranuloma venereum of the vulva in a 24 year old negress developed into a squamous cell carcinoma in the course of 10 months. —H. G. W.

**KELSO, J. W. [Univ. of Oklahoma Sch. of Med., Oklahoma City, Okla.] CARCINOMA OF THE CERVIX. South. M. J., 35:590-593. 1942.**

Clinical considerations are discussed, with emphasis on the use of radium and x-rays. The accomplishments of propaganda are pointed out: "A cancerphobia has never metastasized and has never killed."—H. G. W.

**McNAMARA, F. P. [Finley Hosp., Dubuque, Iowa] HOW NOT TO TREAT CANCER OF THE UTERINE CERVIX. J. Iowa M. Soc., 31:434-436. 1941.**

The inadvisability of treating unexplained uterine bleeding symptomatically is illustrated by a case report. The patient received hormones and small doses of radium, while the cause of an eroded cervix was not investigated. When a hysterectomy was performed at a later date, extensive invasion of the uterine wall from a cancer of the cervix had already occurred.—M. J. E.

**MITCHELL, N., and ANGRIST, A. [Queen's Gen. Hosp., Jamaica, N. Y.] MASSIVE METASTASIS TO BRAIN FROM PRIMARY UTERINE CARCINOMA COMPLICATING AN ADVANCED CASE OF MULTIPLE SCLEROSIS. Am. J. Clin. Path., 12:232-238. 1942.**

Report of the condition described in the title in a 28 year old white woman.—H. G. W.

**SERBIN, W. B. [Chicago, Ill.] CERVICAL POLYPS. Illinois M. J., 80:503-507. 1941.**

While most cervical polyps are harmless, it is well to examine them for microscopic evidence of cancer. Thus, of 6 polyps removed by curettage, 2 contained areas of malignant change. The insertion of radium is indicated if malignancy has supervened.—M. J. E.

**SMITH, F. R. [Memorial Hosp., New York, N. Y.] THE EARLY DIAGNOSIS OF MALIGNANT TUMORS OF THE FEMALE GENITAL TRACT. Illinois M. J., 80:218-222. 1941.**

A general discussion is given.—M. J. E.

**VALK, W. L. [Univ. of Michigan Med. Sch., Ann Arbor, Mich.] UROLOGICAL COMPLICATIONS OF CARCINOMA OF THE CERVIX. J. Urol., 47:686-688. 1942.**

Clinical discussion.—H. G. W.

### Ovary

**BACH, A. C., and MONTGOMERY, M. M. [Cook County Hosp., Chicago, Ill.] RUPTURE OF AN INFARCTED GRANULOSA CELL TUMOR OF THE OVARY WITH MASSIVE HEMORRHAGE. J. Indiana M. A., 34:421-423. 1941.**

Clinically, the condition of a patient with shock and evidence of bleeding into the peritoneal cavity simulated ruptured ectopic gestation. Because of the severity of the symptoms operation was not possible, and a postmortem examination disclosed a twisted, ruptured granulosa cell tumor of the ovary.—M. J. E.

**MARCHETTI, A. A. [Cornell Univ. Med. Coll., New York, N. Y.] OVARIAN CANCER. CLINICOPATHOLOGIC EVALUATION. New York State J. Med., 41:2324-2331. 1941.**

Of the 60 malignant tumors in a series of 1,164 ovarian masses examined, 44 were serous cystadenocarcinomas. Radical extirpation supplemented by roentgen therapy was possible in only 23 cases of the latter group. Eighteen patients died after an average duration of life of 16 months, while 4 still survived after periods averaging 11 months, and 1 appeared cured 7 years after treatment. In the remainder in this group palliative measures consisting of partial removal of the tumor, roentgen therapy, or paracentesis were instituted. Only isolated examples of other types of malignant neoplasms were observed, and the results of therapy were equally discouraging. Of special interest was the cure by operation, generally followed by irradiation, of all 13 patients with histologically benign serous cystadenoma, but with such malignant clinical features as papillary excresences on the external surface of the ovarian mass, rupture of the capsule, or implantations on the peritoneum.—M. J. E.

### Male Genital Tract

**ADAMS, J. E. [Peter Bent Brigham Hosp., Boston, Mass.] A STUDY OF MALIGNANT TESTICULAR TUMORS INCLUDING CASE REPORTS OF CHORIONEPITHELIOMA ACCOMPANIED BY HYPERTENSION AND TERATOMA TESTIS WITH SINGLE TRIDERMAL METASTASES. J. Urol., 47:491-507. 1942.**

A study of 21 malignant testicular tumors lends support to the theory that these tumors, including seminomas and

chorionepitheliomas, have a common origin in spermatogenic cells. Such cells may be pluripotential and give rise to tridermal metastases, as in the case reported. Trophoblastic tissue may be present in teratoma testis more often than is commonly supposed, and this would account for the large amounts of gonadotrophic hormone in the urine of patients with such tumors. The occurrence of hypertension in association with the excretion of large amounts of gonadotrophic hormone in the urine is noted.—H. G. W.

**FRIEDMAN, H. H., and GRAYZEL, D. M. [Jewish Hosp., Brooklyn, N. Y.] MYOMATOUS TUMORS OF THE EPIDIDYMIS. J. Urol., 47:475-481. 1942.**

A case of myoma of the epididymis is reported, with a review of the other 12 cases in the literature.—H. G. W.

**GRAVES, R. C., and LAWRENCE, K. B. [Pondville State Hosp., Walpole, Mass.] BILATERAL EMBRYONAL CARCINOMA OF THE TESTICLE WITH A NOTE ON THE TECHNIQUE OF ORCHIDECTOMY FOR MALIGNANT DISEASE. J. Urol., 47:482-489. 1942.**

Report of an instance of unusually large tumors, weighing 800 and 900 gm., removed by operation.—H. G. W.

**McCOOK, W. W. [Fort Bragg, N. C.] LEIOMYOMA OF THE SPERMATIC CORD. CASE REPORT. Am. J. Surg., 56:499-500. 1942.**

Report of the fourth case, with three others found in the literature. The tumor occurred in a 66 year old negro.—H. G. W.

**O'BRIEN, M. G. [Scranton, Pa.] PRIMARY SARCOMA OF THE EPIDIDYMIS: CASE REPORT. J. Urol., 47:311-319. 1942.**

Only 92 instances of this tumor are recorded. This number includes the author's case, in which trauma seemed to be the etiological factor since the tumor followed 6 months after a violent blow to the scrotal region.—H. G. W.

**TWINEM, F. B. [New York, N. Y.] BENIGN TERATOMA OF THE TESTICLE IN A THREE YEAR OLD BOY: A CASE REPORT. J. Urol., 47:577-579. 1942.**

A case is reported.—H. G. W.

## Prostate

**BALCH, J. F. [Indianapolis, Ind.] CARCINOMA OF THE PROSTATE—NEW METHODS OF MANAGEMENT AND TREATMENT. J. Indiana M. A., 34:672-674. 1941.**

Two examples are recorded of the astonishing clinical and objective improvement which occurs after bilateral orchidectomy in patients with inoperable cancer of the prostate with or without skeletal metastases.—M. J. E.

**BUMPUS, H. C., Jr. [Coll. of Med. Evangelists, Los Angeles, Calif.] CANCER OF THE PROSTATE. California & West. Med., 55:239-241. 1941.**

Prostatic cancers frequently proliferate slowly and not rarely untreated individuals or those who have received only palliative therapy survive many years in acceptable health. These circumstances make difficult an evaluation of therapeutic measures. Tumors are rarely encountered in an early stage, and when sufficiently large to be palpable are generally inoperable, as local extension in the perineural lymphatics about the organ occurs readily. In such cases, however, palliation is indicated, and may prove encouragingly successful. Of 34 patients treated by transurethral resection, 10 survived at least 3 years, 3 at least 5 years, and 1 appeared tumor-free after 7 years.—M. J. E.

**THOMPSON, G. J., and EMMETT, J. L. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE PROSTATE. Surg. Clin. North Am., 21:1181-1187. 1941.**

Malignant lesions of the prostate are discussed under the following headings: incidence, clinical diagnosis, metastasis, conservative surgical treatment, and results of such treatment.—J. L. M.

## Oral Cavity and Upper Respiratory Tract

**FIGI, F. A. [Mayo Clinic, Rochester, Minn.] CANCER OF THE PHARYNX AND LARYNX. Surg. Clin. North Am., 21:1027-1035. 1941.**

This discussion includes the different diagnostic and therapeutic problems of tumors of the nasopharynx, tonsils and oropharynx, base of the tongue and hypopharynx, and larynx.—J. L. M.

## Salivary Glands

**DOCKERTY, M. B., and MAYO, C. W. [Mayo Clinic, Rochester, Minn.] PRIMARY TUMORS OF THE SUBMAXILLARY GLAND WITH SPECIAL REFERENCE TO MIXED TUMORS. Surg., Gynec. & Obst., 74:1033-1045. 1942.**

Of 81 cases, adenocarcinoma, mixed tumor type, was found in 51, cylindroma type in 15, intermediate form in 7, and miscellaneous tumors in 8. There were more females than males in this series. The youngest patient was 12 years old. True mixed tumors are of epithelial origin, are inherently malignant, and should be removed with the salivary gland and regional lymph nodes. A special tendency to invade perineural lymphatic structures, analogous to that occurring with prostatic cancers, is seen here especially with the cylindroma type.—H. G. W.

**McFARLAND, J. [Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.] THE HISTOPATHOLOGIC PROGNOSIS OF SALIVARY GLAND MIXED TUMORS. Am. J. M. Sc., 203:502-519. 1942.**

A long-time study of about 400 mixed tumors of the salivary glands leads to the conclusion that something more than a simple microscopic examination of a section of tissue will be required before an accurate prognosis of a mixed tumor of the salivary glands becomes possible. "At present our methods are no more accurate or scientific than the flipping of a coin." Although the average period between operation and recurrence is 7 years, the fact that it may be much longer, even as long as 45 years, raises the question whether any patient is ever out of danger. The distribution of the mixed tumors in this series, according to origin, was 2 sublingual, 12 submaxillary, and 380 parotid. There were 184 in males and 212 in females, distributed about equally to the right and left sides. The frequency of recurrence of the interstitial tumors was 38% and of the parenchymatous or epithelial tumors 60%, while the highest percentage of recurrence, 81.8, was in the papillary canalicular group. About 50 microscopic slides were submitted to 25 competent microscopists who gave their opinions as to the probability of the recurrence of each type of tumor; 52% of the opinions were correct and 48% were wrong.—H. G. W.

**WOODSON, B. [Temple, Tex.] BENIGN MIXED TUMOR OF THE PHARYNGOMAXILLARY SPACE AND RELATED AREA: REPORT OF A CASE. Texas State J. Med., 37: 785-788. 1942.**

A mixed tumor, possessing the characteristic morphology of the type encountered more commonly in the salivary glands, was extirpated from the pharyngomaxillary space, an area situated internal to the ramus of the mandible and bounded by the deep muscles and fascia of the neck.—M. J. E.

## Intrathoracic Tumors—Lungs—Pleura

**ALEXANDER, J. [Univ. of Michigan Med. Sch., Ann Arbor, Mich.] CIRCUMSCRIBED INTRATHORACIC NEOPLASMS. J. A. M. A., 119:395-397. 1942.**

Contrary to the prevailing impression, the majority of circumscribed intrathoracic neoplasms are intrapulmonary and malignant. Many circumscribed extrapulmonary neoplasms are malignant, and those that are benign may undergo malignant degeneration or cause disability or serious complications as a result of increase in size. Except for those bronchogenic and esophageal carcinomas that yield endoscopic biopsy material and those neoplasms that have metastasized, a preoperative determination of malignancy or benignancy cannot be made with reasonable accuracy. Virtually all presumably removable neoplasms should be operated on promptly. The only proper use of high voltage roentgen therapy is as a therapeutic test for lymphoblastoma or for palliation in inoperable malignant neoplasms.—H. G. W.

**HARRINGTON, S. W. [Mayo Clinic, Rochester, Minn.] PNEUMONECTOMY FOR CARCINOMA OF THE LUNG. J. Thoracic Surg., 11:396-404. 1942.**

A discussion of surgical technic based on 30 cases.—H. G. W.

**HARRINGTON, S. W., and MOERSCH, H. J. [Mayo Clinic, Rochester, Minn.] PRIMARY CARCINOMA OF THE LUNG. Surg. Clin. North Am., 21:1083-1098. 1941.**

Because of the relatively short period of time that carcinoma of the lung has been treated surgically, it is impossible to predict the ultimate place surgery will have in the treatment of this disease. The technical surgical problems at the present time have been solved to a point which makes the operative mortality relatively small. With the improvement in the operative procedures which is constantly taking place, as well as improvement in early diagnosis, it would appear that surgical treatment is the treatment of choice.—J. L. M.

**NEUBUERGER, K. T., and GEEVER, E. F. [Univ. of Colorado Sch. of Med., Denver, Col.] ALVEOLAR CELL TUMOR OF THE HUMAN LUNG. Arch. Path., 33:551-569. 1942.**

A survey of the literature convinces the authors that occasional lung tumors in man, similar to the common lung tumors of mice, arise from the lining cells of the alveolar walls, though they are certainly not more than 5% of all lung tumors. Although the growth is generally called carcinoma, the term "alveolar cell tumor" is to be preferred because of the uncertainty of the histogenesis and the prevalence of the idea that the alveolar lining is not epithelium. This tumor arises as a multiple nodular neoplasm or, less commonly, as a diffuse or lobar infiltrating growth. The incidence in the two sexes is nearly identical, in contrast to the overwhelming predominance of bronchiogenic carcinoma in the male. No metastases were found in approximately half the cases. There are tabulated 25 cases of what seem to be, undoubtedly, alveolar cell tumors, 15 cases that are controversial, and 4 cases of lung tumors with some characteristics of the alveolar cell tumor.—H. G. W.

**NEUHOF, H., RABIN, C. B., and SAROT, I. A. [Mt. Sinai Hosp., New York, N. Y.] A TOPOGRAPHIC CLASSIFICATION OF CANCER OF THE LUNG, WITH SPECIAL REFERENCE TO THE SURGICAL IMPLICATIONS OF THE CIRCUMSCRIBED VARIETY. J. Thoracic Surg., 11:388-395. 1942.**

Gross topographic features were studied with special reference to operability in 281 cases of primary cancer of the lung. Origin from a branch bronchus could be seen in most of the circumscribed tumors which constituted 29% of the series. A bronchial origin could be seen in all cases of noncircumscribed tumors, five-sixths of which originated in large bronchi. Distinctive roentgenologic features existed in the majority of the circumscribed tumors, of which 57% were clinically operable. Twenty-nine of these patients were operated upon: 16 were found free from regional lymph node involvement, while 6 had minimal lymph node involvement. Of the noncircumscribed tumors but 39% were clinically operable; and of 31 patients in this group operated upon, only 9 were free from regional lymph node involvement.—H. G. W.

**OCHSNER, A., and DeBAKEY, M. [Sch. of Med., Tulane Univ., New Orleans, La.] SIGNIFICANCE OF METASTASES IN PRIMARY CARCINOMA OF THE LUNGS. J. Thoracic Surg., 11:357-387. 1942.**

The widespread dissemination of metastases in carcinoma of the lung is indicated by the following percentage figures from 3,047 necropsies: to the regional lymph nodes, 72.2; liver, 33.3; pleura, 29.8; lungs, 23.3; bone, 21.3; adrenals, 20.3; kidneys, 17.5; brain, 16.5; heart and pericardium, 12.7; pancreas, 7.3; peritoneum, 4.8; gastrointestinal tract, 4.3; skin, 3.6; spleen, 3.5; thyroid, 2.3; tonsil, 1.8; tongue, 1.6. Undoubtedly these figures would be raised by more thorough examinations, especially in regard to the metastases in the brain and bones. Two cases are reported in which pneumonectomy was followed by metastases in the axillary nodes, presumably via adhesions from the lungs. The lymphatic drainage of the lungs is briefly described and the various possibilities of metastatic extension by this route are indicated.—H. G. W.

**SANDERSON, R. B. [South Bend, Ind.] CANCER OF THE LUNG. J. Indiana M. A., 34:463-466. 1941.**

A description of 2 cases illustrates the confusing, nonspecific nature of the symptomatology associated with cancer of the lung, and the difficulty of establishing a correct early diagnosis even in those fortunate instances when the patient does not delay in seeking the advice of the physician.—M. J. E.

## Heart

**DOANE, J. C., and PRESSMAN, R. [Jewish Hosp., Philadelphia, Pa.] ANTEMORTEM DIAGNOSIS OF TUMORS OF THE HEART. Am. J. M. Sc., 203:520-524. 1942.**

A case of metastatic carcinoma of the heart secondary to a growth in the uterus, diagnosed clinically, is added to

the 20 recorded in the literature. Of these 20 instances, 13 were sarcomas and 7 carcinomas. In 5 the primary tumor involved the lung. In 9 of the 20 cases the right auricle alone was involved.—H. G. W.

## Gastrointestinal Tract

**COSTEN, J. B., and BRYAN, W. T. K. [Washington Univ. Sch. of Med., St. Louis, Mo.] THE DIAGNOSIS OF CARCINOMA OF THE ESOPHAGUS. Illinois M. J., 80:274-280. 1941.**

The symptoms in patients with esophageal cancer are generally clearly indicative of the lesion, and the clinical diagnosis may be confirmed readily by roentgenographic and esophagoscopic examination. Unfortunately the patients are rarely seen before the growth has extended widely. In some instances, however, patients with the symptoms of the condition may have secondary involvement of the organ, or a nonneoplastic disorder. Thus the authors note examples of cancer of the cardia, larynx, or trachea extending into the esophagus; metastases of more distant tumors situated in the breast or stomach; tuberculosis of the larynx involving the esophagus; and a stricture secondary to adhesions about the organ as a result of an earlier fracture of a lower dorsal vertebra. —M. J. E.

**GILCHRIST, R. K. [Chicago, Ill.] SURGERY OF CARCINOMA OF THE COLON AND RECTUM. Illinois M. J., 80: 249-252. 1941.**

The importance of performing as extensive a resection as possible in patients with operable cancers of the large bowel is demonstrated by the incidence of metastatic foci in 65% of the resected lymph nodes draining the tumor area.—M. J. E.

**GOLOB, M., IPOLITTO, T., and NUSSBAUM, C. [Metropolitan Hosp., New York, N. Y.] CANCER OF THE STOMACH. Rev. Gastroenterol., 9:120-132. 1942.**

An analytic survey of 104 cases, which emphasizes the lack of pathognomic signs and symptoms in early carcinoma of the stomach.—H. G. W.

**GREGG, R. O., and DIXON, C. F. [Mayo Clinic, Rochester, Minn.] OPERABLE MALIGNANT LESIONS OF THE COLON PRODUCING OBSTRUCTION. Surg. Clin. North Am., 21:1143-1152. 1941.**

This paper is based on a review of the records of all patients examined at the Mayo Clinic from 1907 to 1938 who had resectable malignant lesions of the colon, exclusive of the rectum. Of the 2,730 patients examined, 121 had acute obstruction of the colon. Of 76 patients having acute obstruction who survived operation, 75 were traced for a period of at least 5 years. Twenty-six of the 75 patients, or 34.7%, survived longer than this period; only 22.4% of the original 116 who had an operation and could be traced, survived 5 years. This is about 60% of the average survival for all patients examined at the Clinic with lesions in these segments of the bowel, and only about 70% of the average survival rate for those patients recovering from surgical intervention.

The authors believe that obstruction of the colon is associated in some manner with the poor prognosis for patients having malignant lesions of the colon.—J. L. M.

**KISTLER, G. H. [Chattanooga, Tenn.] CARCINOMA OF THE RECTUM. J. Tennessee M. A., 34:390-395. 1941.**

The needs and indications for prompt surgical intervention are stressed.—M. J. E.

**MANHEIM, S., and PESKIN, H. [Mt. Sinai Hosp., New York, N. Y.] SPONTANEOUS ELIMINATION OF A LIPOMA FROM THE SIGMOID FLEXURE. J. A. M. A., 118:1214-1215. 1942.**

A 19th case is added to the literature. The tumor in this instance measured 6 x 4 x 4 cm. and recovery was uneventful.—H. G. W.

**MARKOWITZ, B. [Bloomington, Ill.] BONE CARCINOMATOSIS SIMULATING PERNICIOUS ANEMIA (A CASE REPORT). Illinois M. J., 80:296-298. 1941.**

Necropsy disclosed a cancer of the stomach with diffuse skeletal metastases in a patient who had been considered to have pernicious anemia. Liver therapy had proved valueless.—M. J. E.

**MAYO, C. W. [Mayo Clinic, Rochester, Minn.] MALIGNANT LESIONS OF THE LOWER PORTION OF THE SIGMOID, RECTOSIGMOID, RECTUM AND ANUS, WITH SPECIAL REFERENCE TO ONE-STAGE COMBINED ABDOMINOPERINEAL RESECTION. Surg. Clin. North Am., 21:1129-1141. 1941.**

This article is concerned with the present status of the one-stage combined abdominoperineal resection in cases of malignant lesions of the lower part of the sigmoid, rectosigmoid, rectum, and anus. The author's discussion is based on a review of 179 consecutive cases in which operation was performed. There were 15 deaths in this series. The latter figure includes deaths from all causes, regardless of the time the patients remained in the hospital. In the last 144 cases, the mortality rate was 5.5% and in the last 74 cases there were no deaths.—J. L. M.

**MORRISON, W. A., and DONATH, D. [Los Angeles, Calif.] TUMORS OF THE SMALL INTESTINE. California & West. Med., 55:235-237. 1941.**

Of 45 examples of tumor of the small intestine observed at operation or autopsy, 26 were malignant and 19 benign. The most common type of the former group was adenocarcinoma or fibrosarcoma; of the latter, fibromyoma, adenoma, or carcinoid. Malignant neoplasms generally produced clinical evidence late in their course, and the patients died of metastases or perforation. Benign tumors were either incidental findings or produced intestinal obstruction of gradually increasing severity. The latter could be ameliorated by resection of the mass.—M. J. E.

**RANKIN, F. W. [Lexington, Ky.] MODERN MANAGEMENT OF CANCER OF THE LOWER GASTROINTESTINAL TRACT. Surg., Gynec. & Obst., 74:905-913. 1942.**

A clinical report based on 258 cases with surgical intervention.—H. G. W.

**WALTERS, W., GRAY, H. K., and PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] MALIGNANT LESIONS OF THE STOMACH: RESULTS OF TREATMENT IN THE YEARS 1907 TO 1938 INCLUSIVE. Surg. Clin. North Am., 21:1099-1115. 1941.**

In the years 1907 to 1938, a diagnosis of malignant lesion of the stomach was made in 11,000 cases observed at the Mayo Clinic, and operation was performed in 6,352. These surgical cases are reviewed as to type of lesion, distribution by age and sex, symptomatologic aspects, value of response to treatment as a guide to

malignancy, relationship of physical observations to rate of removability, roentgenologic diagnosis, grade of malignancy and removability of the lesion, treatment and results, mortality and survival rates, and grade of malignancy and prognosis.—J. L. M.

**SCHINDLER, R. [Univ. of Chicago, Chicago, Ill.] THE GASTRIC MUCOSA IN BENIGN ADENOMAS. Am. J. Digest. Dis., 9:149-150. 1942.**

Gastroscopic observations of 36 cases of benign gastric adenomatous polyps lead to the conclusion that atrophic gastritis has a 7 times greater predisposition to the formation of such tumors than has the normal gastric mucosa. However, 15 of 36 patients with adenomatous gastric polyps did not have an atrophic gastritis, as seen gastroscopically.—H. G. W.

## Liver

**GRAY, H. K., and SHARPE, W. S. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE GALLBLADDER, EXTRAHEPATIC BILE DUCTS AND THE MAJOR DUODENAL PAPILLA. Surg. Clin. North Am., 21:1117-1127. 1941.**

This review is based on a study of 464 cases in which operation was performed for carcinoma of the gall bladder and extrahepatic bile ducts at the Mayo Clinic in the years 1907 to 1940.—J. L. M.

## Bone and Bone Marrow

**BEIZER, L. H., HALL, B. E., and GIFFIN, H. Z. [Mayo Clinic, Rochester, Minn.] THE DIAGNOSIS OF MULTIPLE MYELOMA BY STERNAL ASPIRATION. Am. J. M. Sc., 203:829-836. 1942.**

The presence of myeloma cells in bone marrow obtained by sternal aspiration is pathognomonic of multiple myeloma. An increase in the proportion of typical and atypical plasma cells is suggestive, but the absence of abnormal findings does not rule out the possibility of multiple myeloma. Of 10 cases, sternal aspirations confirmed the diagnosis in 8, and in the 9th an increased proportion of the cells were plasma cells.—H. G. W.

**HARPER, F. R. [Denver, Colo.] HUGE CHONDROSARCOMA ARISING FROM THE CHEST WALL AND EXTENDING INTO THE THORAX AND ABDOMEN. J. Thoracic Surg., 11:446-450. 1942.**

A tumor 30 cm. in diameter arose at the site of a trauma.—H. G. W.

**MEYERDING, H. W. [Mayo Clinic, Rochester, Minn.] SOME COMMENTS ON MALIGNANT TUMORS OF BONE. Surg. Clin. North Am., 21:1153-1162. 1941.**

This review discusses types of malignant tumors of bone, their diagnosis, and an evaluation of methods of treatment. At the present time, surgery is unquestionably the most efficient method of treatment in cases of malignant tumor of bone. In order to obtain the best results from such surgical procedures, it is necessary that the patient be seen by a surgeon during the early stage of the disease, that immediate recognition of the type and grade of malignancy be determined by microscopic examination of tissue, and that prompt treatment then be carried out.—J. L. M.

**THOMAS, A. [Denver, Colo.] VASCULAR TUMORS OF BONE. Surg., Gynec. & Obst., 74:777-795. 1942.**

An analysis of the 27 cases in the Bone Sarcoma Registry of the American College of Surgeons. There are 12 cases of benign angiomas and 15 of malignant angiomas, considered to be true endotheliomas. The Ewing tumors are not included. The benign forms possess certain roentgenographic and pathologic features that are practically diagnostic. It is suggested that the malignant angiomas of bone can be divided into angioendotheliomas, which have an epithelium-like structure difficult to distinguish from metastatic carcinoma, and angiosarcomas, which have a greater vasoformative tendency. The malignant angiomas possess few roentgenographic or clinical features distinguishing them from other malignant tumors of bone. They are only moderately radiosensitive, and early diagnosis and radical surgical treatment are essential for cure. —H. G. W.

## Muscle and Tendon

**FULTON, M. N., and SOSMAN, M. C. [Harvard Med. Sch., Boston, Mass.] VENOUS ANGIOMAS OF SKELETAL MUSCLE. J. A. M. A., 119:319-324. 1942.**

In 4 cases of venous angioma of skeletal muscle, diagnosis was made by x-ray determination of phleboliths, which are practically pathognomonic of this tumor when occurring in areas where normally no plexus of veins exists. Injection of radiopaque material is of value in outlining the extent of the tumor. Surgical excision is the treatment of choice but roentgen therapy may be used when extirpation is impossible.—H. G. W.

**GROSS, P., and CAMERON, D. W. [Western Pennsylvania Hosp., Pittsburgh, Pa.] SYNOVIALOMA. Arch. Path., 33: 687-690. 1942.**

About 48 tumors derived from synovia have been reported, to which is added one arising in a suprapatellar bursa. This tumor had been growing for more than 30 years and showed no evidence of recurrence 5 years after surgical removal.—H. G. W.

**HAGGART, G. E. [Lahey Clinic, Boston, Mass.] SYNOVIOMA OF THE KNEE JOINT. J. Bone & Joint Surg., 24: 438-442. 1942.**

Report of a case without recurrence 8 years after removal.—H. G. W.

## Leukemia, Lymphosarcoma, Hodgkin's Disease

**CARY, W. [Finley Hosp., Dubuque, Iowa] SUBLEUKEMIC LYMPHATIC LEUKEMIA WITH SECONDARY ANEMIA. J. Iowa M. Soc., 31:284-285. 1941.**

A diagnosis of lymphatic leukemia was established by examination of the sternal marrow in a patient with severe anemia and only suggestive signs of leukemia in the peripheral blood. The almost complete limitation of the disease to the bone marrow was corroborated by necropsy.—M. J. E.

**HAINES, D. J. [Des Moines, Iowa] ACUTE LEUKEMIA. CASE REPORT. J. Iowa M. Soc., 31:430-432. 1941.**

Fatal myeloid leukemia followed extraction of a tooth. —M. J. E.

# Book Reviews

**SPONTANEOUS AND EXPERIMENTAL LEUKAEMIA IN ANIMALS. By Julius Engelbreth-Holm. Oliver and Boyd, Edinburgh and London. 1942. 245 pages; 44 figures. Price 15 s. net.**

This book was written at the invitation of the Lady Tata Memorial Trust. The Danish manuscript was translated, brought up to date, and edited under the auspices of the Trust, thus taking stock of a period of intensive research which is likely to be interrupted by the present war. The book is carefully written and neatly produced. In the wealth of material surveyed it is comparable to Forkner's "Leukemia and Allied Disorders," and the two works complement each other.

An introductory historical survey is followed by a discussion of the classification and nomenclature of leukemia and related diseases. In his terminology the author adheres to the rule prevalent among hematologists in differing from everyone else, but, to his credit, his classification and nomenclature are refreshingly simple. Many will object to a classification of lymphosarcoma and "reticulosarcoma" under the general heading of leukemia—but not the reviewer. All tumors of myeloid cells are named chloroma. This current trend should be strenuously opposed; not all myeloid tumors are green, and myeloid tumors of chickens are notoriously white.

The description of spontaneous leukemias occupies only a small part of the monograph. Knowledge of leukemia in animals is almost as old as of that in man, and the disease in different species has its own peculiarities and problems. Spontaneous leukemia (leukosis) of fowls is considered in as much space as that of mammals. This is justified by the great economic importance of fowl leukemia and by the peculiarities of this disease not known to those familiar only with human leukemia. For example, erythroleukemia (erythroleukosis), a fairly common disease in poultry, is characterized by a progressive proliferation of basophilic (lymphoid) erythroblasts, and is readily transmitted by a filterable agent. Neurolymphomatosis, one of the most common diseases in poultry, is unknown in man. Its manifestations are sometimes frankly neoplastic, sometimes frankly inflammatory. The relation of lymphomatosis to osteopetrosis, recently recognized by Jungherr, is, of course, not considered. Nor are the recent successes of Olson and of Pentimalli in transmitting common lymphomatous tumors, a much debated point in the older literature.

As for the larger mammals, leukemia in cattle is of some economic importance. It has been studied extensively, and the suggestion made that heredity plays a major role in its occurrence, but adequate proof is lacking. The gross and microscopic changes and the blood picture of the different forms of leukemia are amply illustrated.

Almost all aspects of experimental leukemia are described and the findings, whether of the writer or of others, are discussed objectively. The material is well organized, and with the aid of the contents and of the index one can learn about the different aspects of the subject as little or as much as one desires. Under the heading Transmission of Fowl Leukemia are considered the character of the different strains, variations in percentage of takes, specificity of the strains, spontaneous remission and recovery from the transmitted disease, relationship between fowl leukemia and sarcoma, and the interrelationship of the different forms of leukemia. The various transmissible strains have their own well defined characteristics and their specific cell affinities are constant. The recent report of Duran-Reynals which contradicts this thesis is not considered but this work, revolutionary if correct, needs confirmation. Like Ellerman, discoverer of the virus of fowl leukosis, Duran-Reynals may not have appreciated the frequency of the spontaneous occurrence of the different types of leukosis among poultry and the variability of this frequency in different stocks and at different times. However, it is now generally conceded that leukosis and sarcoma are related. Not only are their properties alike but one virus may produce both. The author prefers the term virus to the less committal term agent, even though he has been considering the possibility that this virus is endogenous in origin. The tissue culture studies center around this question—can these viruses be cultivated *in vitro* only in the presence of cells which they endow with malignant properties *in vivo?* The few immunity studies reported point further to a similarity between sarcoma and leukosis. Uhl, an associate of Engelbreth-Holm, succeeded in immunizing chickens actively against fowl leukosis and has correlated immunity with the presence of neutralizing antibodies in the sera.

The chapter on transmission of mammalian leukemia includes studies on the lowering of resistance by irradiation with x-rays, attempts to demonstrate a virus in mouse leukemia, and metabolic changes in leukemic tissues. The statement is made that Krebs and his associates "found a means of lowering the resistance of mice to transplanted tumors by x-rays," but these authors themselves duly credit James B. Murphy with this discovery.

The description of experimental findings is everywhere complemented by a discussion of the fundamental problems, most of which can be answered only by further experimentation; as, for instance, why is there a rise of virulence in the course of successive passages of transmission lines, and has cell-free material actually produced leukemia or merely hastened the development of the disease in susceptible animals? Throughout the book there are "hints of phenomena about the nature and significance of which we can only guess."

The section devoted to heredity wisely considers the spontaneous and transmitted disease separately. There are two additional papers now on record concerning the heredity of spontaneous mouse leukemia (Mercier, Cole

and the reviewer), and these allow one significant conclusion to be appended; namely, that the mode of inheritance of leukemia varies with different stocks and that different types of leukemia may have different genetic bases. Considering the rapid progress made recently in the study of extrachromosomal factors, this section is fairly up to date. However, a few corrections are needed. Foster nursing does influence the occurrence of leukemia, and female mice have a higher incidence of the disease than males. The milk factor of Bittner is considered to be a virus, and reasons are given why mouse leukemia may likewise be produced by a virus, even though experimental studies have thus far failed to provide a solid basis for this theory.

The section on the significance of heredity in transmissible leukemia includes a survey of immunity reactions and their dependence upon heredity. Perhaps too much space is given to "Potter's theory." Evidently Engelbreth-Holm is anxious to consider every clue which might elucidate the phenomena of experimental leukemia. Potter's theory was evolved in studies on immunity, and this theory, "though not improbable, certainly fails to offer an immediate explanation of the involved features of the immunization experiments described." The nature of the immunizing processes is vividly surveyed in the light of Gorer's recent experiments.

In the chapters on the experimental production of leukemia in both fowls and mammals, leukemoid reactions and leukemia are clearly distinguished, and the production of neoplasms other than leukemia by similar agents is harmoniously considered. Engelbreth-Holm adheres to the view that the production of leukemia by carcinogenic hydrocarbons is essentially an acceleration of a normal tendency. The reviewer is with the minority in considering the possibility of a different mechanism involved in hereditary and induced leukemias. How else could be explained a decided difference in the genetic character of the malignant cells in spontaneous and induced leukemias of the same inbred stock of mice?

In the closing chapter the nature of animal leukemia is considered and the conclusion reached that it is a malignant disease of the hemopoietic tissues. The statement made over and over again that there is a fundamental difference between leukemias in mice and in men is convincingly refuted. The similarity in behavior is remarkable; indeed, "so perfect right down to the smallest detail that doubt as to the identity of the two diseases can hardly be entertained."

JACOB FURTH

**THE DYNAMIC STATE OF BODY CONSTITUENTS. By Rudolf Schoenheimer. Harvard University Press, Cambridge, Massachusetts. 78 pages; 6 figures and 16 tables. Price $1.75.**

This book is made up of three Edward K. Dunham lectures which were given at Harvard University in the fall of 1941. Owing to the untimely death of Dr. Rudolf Schoenheimer, the lectures were prepared for publication by Drs. H. T. Clarke, D. Rittenberg, and S. Ratner. The lectures—The Reactions of the Body Fats Investigated with Deuterium, The State of the Body Proteins, and The Role of Structural Elements in the Formation of Excretory Products—review and interpret the work on the intermediary metabolism of body constituents carried out so brilliantly with the aid of isotopes (heavy hydrogen and heavy nitrogen) by Dr. Schoenheimer and his collaborators. As a result of these investigations the author suggests the general concept "that all constituents of living matter, whether functional or structural, of simple or of complex constitution, are in a steady state of rapid flux." To support this concept many experiments are cited on the metabolism of fatty acids, amino acids, and excretory products which show that these molecules, when supplied in the diet, rapidly interchange with and replace chemically identical molecules of the body tissues. At the same time they undergo numerous other transformations. Many of these transformations are fundamental chemical reactions proceeding continuously in the animal organism regardless of the constituents of the diet. To illustrate this general thesis, it was found in studies on fat metabolism that on feeding palmitic acid to adult rats of constant weight the dietary fatty acid not only exchanged with the palmitic acid of the storage fat but also was converted into other fatty acids. In experiments on protein metabolism, glycine fed to animals in nitrogen equilibrium replaced glycine in structural as well as functional proteins and the nitrogen from the dietary amino acid was utilized for the formation of other amino acids. The conversion of phenylalanine into tyrosine in the body, even in the presence of an excess of dietary tyrosine, is an illustration of the concept that many chemical reactions are not dependent on the constituents of the diet.

Dr. Schoenheimer and his collaborators, by employing molecules labeled with isotopes, were able to investigate a number of problems in intermediary metabolism which had previously defied a direct approach. The book deals with the valuable contributions to biochemistry made by his group and will prove of great interest to students of the biological sciences.

DAVID SHEMIN